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The current research was designed to examine the effects of acute and recurrent stress during late adolescence on subsequent indices of adult behavioral health. The research used an animal (rat) model to examine four specific aims: (1) determine how repeated acute predator stress during adolescence affects behavioral indices of anxiety, depression, and alcohol consumption during adulthood; (2) evaluate how recurrent sleep disruption during adolescence affects behavioral indices of anxiety, depression, and alcohol consumption during adulthood; (3) evaluate the combined effects of predator stress and sleep disruption during adolescence on behavioral indices of anxiety depression, and alcohol consumption during adulthood; (4) evaluate genetic and sex differences in the stress effects during adolescence on adult behavioral indices of anxiety, depression, and alcohol consumption during adulthood in male and female rats of two different strains (genotypes).

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Effects of acute and recurrent stress during adolescence on subsequent indices of adult behavioral health in rats

by

Michael E. Perry

Doctoral Dissertation Proposal submitted to the Faculty of the
Department of Medical and Clinical Psychology
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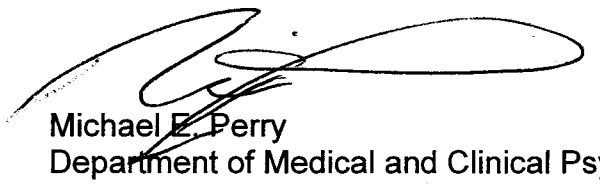

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ABSTRACT

Title of Thesis: Effects of acute and recurrent stress during adolescence on subsequent indices of adult behavioral health in rats

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The current research was designed to examine the effects of acute and recurrent stress during late adolescence on subsequent indices of adult behavioral health. The research used an animal (rat) model to examine four specific aims: (1) determine how repeated acute predator stress during adolescence affects behavioral indices of anxiety, depression, and alcohol consumption during adulthood; (2) evaluate how recurrent sleep disruption during adolescence affects behavioral indices of anxiety, depression, and alcohol consumption during adulthood; (3) evaluate the combined effects of predator stress and sleep disruption during adolescence on behavioral indices of anxiety, depression, and alcohol consumption during adulthood; (4) evaluate genetic and sex differences in the stress effects during adolescence on adult behavioral indices of anxiety, depression, and alcohol consumption during adulthood in male and female rats of two different strains (genotypes).

The research was divided into two experiments. Experiment 1 established the feasibility of conducting an experiment utilizing predator stress and sleep disruption as adolescent stressors. Experiment 2 used both stressors to determine the effects during adolescence on indicators of adult behavioral health in male and female Sprague-Dawley and Long-Evans rats. In both experiments, the independent variables were: no stress, predator stress, sleep disruption, and predator plus sleep disruption (combined). In Experiment 2, the independent variables also included sex and genetic strain. The dependent variables in both experiments were serum corticosterone, open field activity (including center time as index of anxiety), forced swim immobility (index of depression), and voluntary alcohol consumption.

There were sex, strain, and condition differences. Rats in stress conditions displayed higher corticosterone levels than controls. Rats in the sleep condition also displayed greater anxiety-like behavior, with females more anxious than males. SD rats displayed more depression-like behavior (forced swim immobility) regardless of condition, and males generally displayed more depression-like behavior than females, with SD males displaying significantly more depression-like behavior than all other groups. SD rats consumed more alcohol overall than LE rats. Animals in the sleep disruption condition consumed more alcohol than other groups. The results revealed that stress during adolescence, particularly sleep disruption, has long-lasting effects well into adulthood in rats.

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Introduction

Overview

Military personnel exposed to combat environments are at risk for profound mental health problems (Hoge et al., 2004). The majority of troops deployed to Operation Iraqi Freedom/Operation Enduring Freedom (OIF/OEF) indicate that they have experienced severe stressors such as: attack or ambush by the enemy, incoming rocket fire, and/or incoming small arms fire (Hoge et al., 2004). Other stressors encountered by deployed forces include long work hours and time pressure leading to lack of adequate sleep (Kavanaugh, 2005). The 2005 Survey of Military Health Behaviors revealed deployment, increase in workload, and separation from family to be among the greatest stressors for military personnel (Bray et al., 2006). OIF and OEF have generated a 70% increase in military veterans seeking mental health treatment for stress-related illness, making mental illness the second most treated injury among Iraq and Afghanistan veterans (Zoroya, 2007). One fourth of OIF/OEF veterans seen at VA health care facilities are reported to have received mental health diagnoses, with over half receiving two or more distinct diagnoses (Seal et al., 2007). Post-deployment mental health screening of service members deployed to OIF/OEF revealed that depression and anxiety were the two most highly endorsed mental health symptom clusters (Hoge, 2004).

The majority of individuals deployed with combat units in Operation Iraqi Freedom (OIF) and Operation Enduring Freedom (OEF) are between the ages of 18 and 24 years of age. Adolescence is broadly considered to be the period of

life between the beginning of sexual maturation (puberty) and adulthood, generally between ages 13 and 19 (Marshall, 2006). The National Center for Education in Maternal and Child Health recognizes adolescence as between 11 – 21 years of age (Green & Palfrey, 2002). Based on ineffective coping and unemployment problems observed among Vietnam veterans, Vinokur et al. (1987) suggested that pre-war stress and combat stress had additive and deleterious effects on emotional well-being. Meadows et al. (2006) indicate that stress experienced by some adolescents might increase the probability of depressive symptoms as they grow into adulthood, and that support from parents might have an attenuating effect. Kaplow & Widom (2007) found that maltreatment during childhood (e.g., physical and sexual abuse, neglect, etc.) predicted long-term mental health problems, particularly anxiety and depression, later in adulthood. War-related trauma during childhood—particularly, death of family members, displacement, destruction of home, or witnessing death—has been linked to lasting affective and anxiety-like symptoms including aggression, emotional lability, hyperactivity, and social withdrawal in a population of Lebanese 6-9 year-olds (Chimienti, Nasr, & Khalifeh, 1989). High school and university students in high-risk, frequently targeted areas of Lebanon (Tel Aviv and Haifa) during the first Gulf War reported symptoms including persistent fear, re-experiencing, avoidance of pleasant activities, and difficulty concentrating (Klingman, 1992). This information is particularly relevant to young, deployed military personnel when one considers that Posttraumatic Stress Disorder (PTSD) cases can emerge long after personnel have left combat. In a study

conducted by the Veteran's Administration, nearly 20% of a representative sample of Vietnam veterans developed lifetime PTSD and nearly 10% were suffering with PTSD 10-11 years after the war (Dohrenwend et al., 2006).

Of the human (i.e., Meadows et al., 2006; Tyano et al., 1996; Seal et al., 2007; Toomey et al., 2007) and animal (i.e., Andersen & Teicher, 2004; Imanaka et al., 2006; Romeo et al., 2006) studies that have examined the effects of early stress on adult mental health, few have examined adolescence *per se*, although Seal and colleagues (2007) identify deployed 18 – 24 year old veterans as the group at highest risk for post war psychopathology when compared to older veterans. The studies investigating the effects of stress during younger years on subsequent mental health in a deployed military population are limited (Seal et al., 2007; Toomey et al., 2007; Vinokur et al., 1987) and reports are primarily related to deployment. Questions remain as to how specific stressors in deployed environments might affect the individual years after deployment.

This doctoral research project used a rodent model to examine effects of stressors (acute and recurrent) during late adolescence on subsequent behaviors during adulthood that are indices of anxiety, depression, and alcohol consumption. The particular stressors (predator stress and sleep deprivation) used in the present research modeled stressful exposures experienced by active duty military personnel. As background for this project, this paper summarizes effects of stress on military service members and on health and behaviors relevant to health. The next section provides a rationale for the use of animal models to answer questions related to stress in humans, with an explanation of

the stressors chosen for manipulation in the current research project. After the presentation of the rationale, an overview and specific aims of the conducted research is presented. Following the general overview, the details of Experiment 1 are presented, followed by Experiment 2, including experimental overview, hypotheses, methods, results and discussion for both experiments. The methods sections provide detailed descriptions and rationale for how the experiments were conducted and how the data were analyzed.

Stress

Stress occurs when environmental demands challenge or exceed one's capacity to adapt (Cohen et al., 1995). While initially adaptive, stress can ultimately exact a costly psychological and physiological toll if left unchecked. This psychobiological perspective of stress and its effects emerges from over a century of research focused first on biology, then psychology, and evolving into an integrated concept. Although beyond the intended scope of the research conducted, a history of stress research is presented in Appendix B to elucidate foundational aspects of the current research.

Effects of Stress on Military Service Members

The United States military is made up of a diverse group of members, with many races/ethnicities, men, women, and age groups represented (GAO, 2005). Because of the nature of the military's mission, stress can emerge in numerous forms, including: extreme heat, irregular lighting, sleep loss, threat of injury or death, or time pressure to meet deadlines or accomplish missions (Kavanaugh, 2005). During U.S. deployments to Haiti, Bosnia, Somalia, and Kuwait in the

1990s, the most commonly reported stressors included: separation from family, uncertainty, poor sanitation, lack of privacy, long work hours, fear of disease, lack of sleep, and family and financial problems (Kavanaugh, 2005). Time in the theater of operations and workload are both thought to increase stress levels (Halverson et al., 1995). Factors impacted by stress can vary depending on the location and mission objectives. Because the *exact* factors responsible for stress effects in a deployed setting are difficult to isolate, the present research targeted two stressors with support in the research literature that are relevant to military situations: predator stress and sleep.

Combat Exposure

The wars being fought in Iraq and Afghanistan are producing a generation of military veterans who are at risk for chronic mental health illness secondary to trauma exposure and hardship of an active and complex combat theater (Litz, 2007). In post deployment health assessments from current operations in Iraq and Afghanistan, 58% of Army soldiers in Afghanistan, 89% of Army soldiers in Iraq, and 95% of Marine Corps personnel reported being shot at by the enemy or ambushed (Hoge et al., 2004). Hoge and colleagues reported that personnel deployed to Iraq and Afghanistan endorsed significantly higher rates of symptoms related to mental disorders, particularly anxiety, depression, and PTSD when compared to undeployed personnel. Similarly, sources of profound stress during Operation Desert Storm included threat of enemy fire, managing casualties, and handling human remains (Adler et al., 1996; McCarroll et al.,

1993). When considering attacks by small-arms fire, even more Army and Marine Corps personnel reported having been targeted by the enemy.

It is clear that being targeted and shot at by the enemy in a war zone creates an unpredictable and stressful environment, characterized by fear of injury or death. Therefore, an animal model—predator stress by presentation of a predator's scent in a novel environment—was used to simulate the conditions described above and to model the environment experience by deployed soldiers.

Sleep

Stress for military personnel exists in environments beyond the direct combat experienced in the Persian Gulf region. Peacekeeping operations and even daily military training and garrison operations can produce high levels of stress with adverse effects on functioning (Johnson et al., 2007). Among the stressors reported most problematic in peacekeeping operations is sleep deprivation or disruption (Halverson et al., 1995), which can degrade the ability to make clear decisions and further exacerbate levels of stress (Larsen, 2001). As technology has advanced, work duration previously restricted by limited night vision is now limited only by endurance, which is directly impacted by amount of sleep obtained (Giam, 1997). Based on observation of Army Ranger School candidates in training and units rotating through the National Training Center, Belenky (1997) reported that the consequences of inadequate sleep were "reduced individual and unit effectiveness, errors, accidents, increased casualties from enemy action, and friendly fire incidents" (p. 12). Lieberman et al. (2005) studied members of an elite Army Infantry unit whose members remained active

and slept only three hours per night during a 53 hour training exercise. Lieberman and colleagues observed profound reaction time, attention, memory, and reasoning decrements. With regard to sleep in the military, a survey of health behaviors in the past 12 months revealed that over 18% of Army personnel reported getting an average of three to four hours of sleep each night. Sixty-four percent of Army personnel reported obtaining five to six hours of sleep each night, but only about 16% reported getting seven or more hours of sleep each night. Of the individuals in the Army obtaining the least sleep, those 20 years old or younger represent the largest proportion of the force (Bray et al., 2006).

In the short term, sleep loss in humans has limited adverse physiological consequences beyond sleepiness and impaired performance with some tasks (Sluyters et al., 2003). There is evidence that prolonged sleep disturbance, such as that produced by noisy environments or chronic restriction of sleep, affects slow-wave sleep and rapid eye movement (REM) (Kawada & Suzuki, 1999), impairing the recuperative value of sleep episodes. Chronic restriction of six or less hours of sleep for 14 nights can produce cognitive deficits comparable to two nights of total sleep deprivation, including deficits in attention, working memory, cognitive throughput, and behavioral alertness (Van Dongen et al., 2004).

Animal research has reported stressful effects of sleep disturbance and deprivation. Although effects of sleep disturbance vary based on the extent of sleep loss, total sleep deprivation can result in loss of body mass, ulcerative skin

lesions, hyperphagia, hypothermia, septicemia, and death in rodents subjected to total sleep deprivation (Everson, 1995; Rechtschaffen et al., 1983).

Sleep is a relevant variable in the proposed research, because it is established in the human, military, and animal literature that sleep loss can be detrimental to mental and physical health, particularly mood and anxiety disorders (Breslau et al., 1996). Therefore, sleep disruption was used as a stressor in the present research.

Past Stress and Subsequent Behaviors

The effect of acute and recurrent past stress on subsequent behaviors and health in active duty personnel and veterans has been an area of research interest since at least the Vietnam era. In a sample of male, Vietnam veterans, Vinokur and colleagues (1987) reported that exposure to war produced long-standing adverse effects on emotional well-being in unemployed veterans. Vinokur et al. indicated that stressful childhood and adolescent experiences might have long-standing effects on mood, anxiety, self esteem, and life satisfaction. Cohen et al. (2007) suggested that exposure to traumatic stimuli early in life significantly increases the risk of high stress reactivity during adulthood.

With regard to military deployment-related stressors, Toomey et al. (2007) reported that the overall prevalence of depression and anxiety fell in both deployed and non-deployed Gulf War veterans 10 years after the war. Despite the decline, deployed veterans experienced higher levels of psychological distress and lower quality of life than did the non-deployed group, even after 10

years. As previously mentioned, Vinokur et al. (1987) established that experiences before, during, and after war were associated with impaired mental health function later in life. Further, a recently released report found that active duty personnel and veterans endorsed mental health concerns at higher rates during a six month post deployment re-assessment than they did on the initial assessment conducted immediately after deployment (Milliken, 2007). Similarly, 78% of a sample of Army soldiers injured in combat screened positive for Posttraumatic Stress Disorder (PTSD) and depression at Walter Reed Army Medical Center at a seven month assessment after having previously screened negative at one month after admission (Grieger et al., 2006). It is unclear what the mental health disorder prevalence will be for these individuals in the coming years and months.

The extent to which stressors during adolescence and early adulthood affect behaviors and health in later adulthood remains unclear. The types of stress during adolescence which might have later effects also are not well established. If stressors related to deployment and combat, such as awaiting attack or limited sleep, have significant effects later in life, then the results for current and former military personnel could be staggering. With an estimated 17% of personnel exposed to combat electing to separate from service after deployment (Hoge et al., 2006), there might be substantial numbers of affected individuals outside of the umbrella of military health care.

Effects of Stress on Health

Stressors can have profound effects on physical and mental health that vary based on a number of factors, including the duration of the stressor, number of stressors, appraisal of the stressors, premorbid health, sex, age, and underlying genetic factors of the individual under stress (McEwen, 1998; Lazarus, 1998; Schneiderman et al., 2005). Probably the most damaging feature of a stressor is the duration of the stressor (McEwen, 1998; Selye, 1975). Acute responses to stressors are considered adaptive and beneficial for the organism in the short term, whereas chronic responses to stressors are generally considered as harmful for the organism. This information becomes important when considering the effects of the various stressors in deployed military environments. Many military-related stressors can be categorized as acute or chronic.

Acute Stress Response

The acute, adaptive response occurs after the perception of a stressful event and involves changes in the nervous, endocrine, and immune systems. Selye (1956) reported that the bodily responses to stressors are generally adaptive in the short term in two major ways: release of stress hormones to release energy stores for immediate use, and changes in the pattern of energy use. Under acute stress, resources are diverted to tissues and organs that must respond quickly to stressors, particularly to the brain and skeletal muscles.

Stress-induced endocrine responses provide a mechanism for stress responses stemming from the actions of the hypothalamic-pituitary-

adrenocortical axis (HPA) and the sympathetic-adrenomedullary (SAM) systems (Cohen et al., 2007). Activation of the HPA axis mobilizes cortisol, a stress hormone which is instrumental to anti-inflammatory responses; fat, carbohydrate, and protein metabolism; and gluconeogenesis. The SAM works in concert with the HPA, releasing catecholamines which are agents of the autonomic nervous system helping to regulate the cardiovascular, immune, pulmonary, hepatic, and skeletal muscle systems (Cohen et al., 2007). Blood levels of cortisol (in humans) and corticosterone (in rats) provide indices of human stress responses (Grunberg & Singer, 1990; McEwen, 2000)

The immune system also is activated during acute stress, redistributing leucocytes from the blood into the organs and tissue (Dhabhar & McEwen, 1997; Schneiderman et al., 2005). Leucocytes are responsible for defending the body against infectious diseases and foreign substances. From an evolutionary perspective, activities that are less critical are suspended during stressful situations. Less critical functions (in the short run) include digestion and production of growth hormones or gonadal hormones (Schneiderman et al., 2005).

Chronic Stress Response

If chronically activated, the acute stress response can become maladaptive (Selye, 1956). Chronic and damaging response to stressors, or allostatic overload, apply what McEwen (1998) called “wear and tear” on the body as it attempts to adapt to an ever-changing environment. Cortisol and adrenalin (epinephrine), which serve to restore energy in acutely stressful

situations, promote fat deposition, insulin resistance, hypertension, and cardiovascular ailments when the body is unable to utilize the energy it obtains from food, alternatively storing it as adipose tissue (McEwen, 2001b).

Cortisol and adrenalin are responsible for mobilizing cells of the immune system during acute stress responses, but cause immunosuppression when stress is repeated or chronic (McEwen, 2001; Schneiderman, 2005), placing the individual at increased risk for infectious disease. Henry and colleagues (1975) also reported that chronic, stress-induced activation of the sympathetic activation of the cardiovascular system increases blood pressure and vascular hypertrophy, a thickening of vascular structure.

Chronic exposure to stressful situations also has effects on mental health. McEwen (2001b) reported that the effect of cortisol and adrenaline on the hippocampus promotes memory formation associated with harmful experiences in the short term, protecting the individual from future hazards. However, prolonged exposure to stress hormones often results in neuronal atrophy resulting in memory impairment, as well as growth of neurons in the amygdala, enhancing fear responses (McEwen, 2004).

Karasek and Theorell (1990) reported that high demand combined with low control resulted in a higher level of stress than either of those factors alone. Multiple factors involved in stress might work together to produce a more potent stress effect, such as the effect of time pressure combined with threat in work stress (Stanton et al., 2001).

It is clear that the impact of acute and chronic stress responses is relevant with regard to military operations, especially in operational and deployed environments. The additional consideration of multiple stressors is an important factor as well, given that time pressure, mission requirements, and other environmental stressors are associated with military operational work. It is unclear, however, what specific stressors have the greatest effects in what individuals.

Physiological Effects

Protracted or recurring activation of the HPA and SAM can disrupt their ability to regulate other physiological systems and increase risk for physical and mental disorders (Cohen et al., 1995; McEwen, 1998). Animal research provides strong support for coronary artery disease resulting from stress exposure, mediated by prolonged SNS activation (Rozanski et al., 1999). Experimental work also reveals that stress might induce pathogenic cardiac processes (Krantz & McCeney, 2002); hasten HIV/AIDS progression (Vedhara, 2005); and contribute to the initiation, growth, and metastasis of some cancerous tumors (Antoni et al., 2006), although evidence linking stress and cancer incidence in humans remains mixed (Duijts et al., 2003; Heffner et al., 2003; Turner-Cobb et al., 2001). Reports indicate that stress increases pro-inflammatory cytokines, the proteins responsible for immune and inflammatory function, inhibit the clearing of viruses, and disrupt the inflammatory process (Meagher, 2007; Miller et al., 2002). Chronic stress might exacerbate inflammation and increase risk for

development of central nervous system infection, neurodegenerative diseases, and inflammatory diseases (Meagher, 2007).

Behavior

Behavioral changes, as attempts to cope with stress, can create risk factors for disease. Increased tobacco smoking, increased alcohol consumption, decreased exercise, poor sleep, and lack of adherence to prescribed medical regimens are common ways in which individuals' respond to stress and place themselves at greater risk for disease (Bray et al., 1999; Heffner et al., 2003; Krantz & McCeney, 2002). The American Psychological Association commissioned an online survey of over 1800 adults between August 30 and September 11, 2007, to examine the state of stress across the United States (APA, 2007). The results revealed that, when experiencing high levels of stress, 67% of cigarette smokers smoked more when stressed; 17% of alcohol drinkers increased alcohol intake; nearly 50% of adults were unable to obtain sleep; over 40% of adults overate or consumed unhealthy foods; and over 33% of survey participants skipped a meal because of a stressful period or event (APA, 2007).

The extent to which stress actually promotes disease, motivates treatment seeking behavior, or both, remains unclear. However, it is clear that exposure to chronic stress is generally considered most harmful, because of long-standing or permanent changes in emotional, physiological, and behavioral responses that impact disease risk and course of illness (Baum et al., 1983; Baum et al., 1992; McEwen, 1998).

With regard to the current research project, the conditions of interest include mood states, anxiety, and alcohol consumption. Depression, Generalized Anxiety Disorder (GAD), Post Traumatic Stress Disorder (PTSD), and alcohol abuse are the most highly endorsed mental health related conditions among redeploying, active duty personnel from the current wars in Iraq and Afghanistan. The same pattern of conditions exists in separated personnel entering the Veteran's Affairs (VA) Health Care System (Hoge et al., 2004; Milliken et al., 2007; MIRECC, 2006).

Depression

Stressful life events are likely to precede the onset of major depressive episodes in patients when compared to controls (Hammen, 2005). Common symptoms of depression can include: persistent sadness or despair; changes in appetite; psychomotor retardation; anhedonia; apathy, low motivation, social withdrawal; difficulty with concentration or memory; low energy level; hopelessness; low self-esteem; or suicidal ideation (APA, 2000). Experiencing five or more symptoms of depression during a discrete period of two or more weeks is defined as a major depressive episode (MDE). At least one of the five symptoms must be sadness or loss of pleasure. Major Depressive Disorder (MDD) is defined by two or more MDEs separated by less than two months between episodes.

For individuals between the ages of 15 and 44, Major Depressive Disorder (MDD) is the leading cause of disability in the United States (WHO, 2004). Based on a nationally representative survey of 5692 individuals, approximately

6.6% of adults in the United States are estimated to suffer from MDD every year, and lifetime prevalence is approximately 16.2% across the population (Kessler et al., 2007). Even though most individuals are not diagnosed with MDD, depressive symptoms remain a factor in the lives of many individuals. A survey on stress in the United States by the American Psychological Association (APA) suggests that a substantial proportion of the population experiences some of the symptoms of depression, although there might be no clinical diagnosis. According to the survey, 45% of respondents reported anergia and 36% reported sadness in the previous month. Mental health disorders can occur at any age, but the median age of MDD onset is 32 years old (Kessler et al, 2007).

Evidence exists to suggest that episodic stressors have a causal role in the instance of major depression. Post et al. (1992) hypothesized that repeated stressors result in neurobiological changes which lead to recurrent mood episodes. According to the hypothesis, an individual becomes sensitized as a result of the neurobiological changes, and the mood disorders gradually become independent of the stressors, resulting in increased likelihood of spontaneous mood episodes. In support of Post and colleagues, Kendler et al. (2000) reported a diminishing association between stressful life events and depression as the number of depressive episodes increased. A prospective study of college students recruited in the mid 1940s and followed for 40 years revealed that negative life events affected psychological health, particularly affective disorders, more than physical health (Cui & Vaillant, 1996).

The vast majority of research supporting a stress-depression relationship is based on discrete episodes of stress and not necessarily chronic stressors (Hammen, 2005). Of the limited studies to investigate both episodic and chronic stressors, Rojo-Moreno and colleagues (2002) reported that depression was equally predicted by acute and chronic stressors in a clinically-depressed population. Hammen and colleagues (1992) also found support for a relationship between episodic and chronic stressors and their effect on depression.

Anxiety

Similar to depression, stressful life events also precede anxiety disorders (Faravelli & Pallanti, 1989; Finlay-Jones & Brown, 1981). Anxiety disorders in the *Diagnostic and Statistical Manual* (DSM-IV-TR; APA, 2000) include Acute Stress Disorder, Generalized Anxiety Disorder, and Posttraumatic Stress Disorder (APA, 2000). Stressful events that involve dimensions of loss, humiliation, and danger have been found to be related to the development of generalized anxiety disorder (GAD) and major depression (Kendler et al., 2003). The symptoms of GAD include excessive worry that is difficult to control and at least three of the following six symptoms: restlessness, fatigue, concentration problems, irritability, muscle tension, or sleep disturbance (difficulty falling asleep, staying asleep, or unsatisfying sleep) (APA, 2000). The most recent National Comorbidity Survey Replication (NCS-R) survey reported a 12 month GAD prevalence of 3% and a lifetime prevalence of 5.6% (Kessler et al., 2007) in a nationally representative sample of 5692 adults. The 2007 *Survey of Stress in America* (APA, 2007) revealed high self-reported rates of symptoms related to

anxiety. Kessler et al. (2005) also reported high self-reported rates (18.1%) of anxiety among Americans.

The NCS-R reported overall lifetime prevalence of PTSD of 6.8%. This is a relatively low rate when one considers that 60% of men and 51% of women reported being exposed to at least one traumatic event (Keane et al., 2006). There are some sub-populations, however, that face a higher-than-normal probability of exposure to a life-threatening situation and higher than average rates of psychopathology. For example, among a group of 21 to 30 year old Detroit area individuals, 40% reported exposure to a traumatic event, and 9.5% met criteria for PTSD. Approximately 11.5% of former public school students in Miami-Dade County met criteria for lifetime PTSD. Military members in Iraq, by comparison, are exposed to potentially traumatic stressors at a rate of over 90% (Hoge, 2004).

Posttraumatic stress disorder is characterized by experience or exposure to a fearful, traumatic, or life threatening event and three symptom clusters (APA, 2000). The three clusters are one or more symptoms of re-experiencing of the trauma; three or more symptoms of persistent avoidance of trauma-related stimuli; and two or more symptoms of persistent arousal. Re-experiencing symptoms might include intrusive thoughts, recurring dreams, flashbacks, or reactivity upon exposure to symbolic cues resembling some aspect of the traumatic event. Avoidance of traumatic stimuli include active effort to avoid thoughts, feelings, or conversation related to trauma; emotional numbing or detachment; restricted range of affect; avoidance of places or activities

reminiscent of trauma; inability to recall key elements of traumatic situation; diminished interest in important activities; or a sense of a foreshortened future. Symptoms of arousal include difficulty sleeping; irritability or anger; concentration problems; hypervigilance; or exaggerated startle response (APA, 2000).

Comorbidity of Depression and Anxiety

Depression and Anxiety are highly comorbid conditions. A recent report of mental health diagnoses issued to discharged military personnel at the Puget Sound VA Health Care System indicated that over one third of patients were given two or more diagnoses (MIRECC, 2006). Although depression and anxiety are currently classified as separate disorders with distinct symptoms, there are some features that frequently overlap and co-occur between the disorders (Mineka et al., 1998). Of the individuals meeting lifetime criteria for MDD, 59% are diagnosed with a comorbid anxiety disorder at least once in their lifetime (Kessler et al., 2007). In a 12 month period, over 57% of individuals diagnosed with MDD have a comorbid anxiety disorder of some sort. There is evidence suggesting that the onset of anxiety precedes the onset of depression in most cases (Kessler et al., 1997; Lepine et al., 1993; Mineka et al., 1998). The classification of depression and anxiety as separate disorders has been routinely debated in the research literature (Lillienfeld, 1994), with some researchers advocating for the current approach of recognizing two distinct disorders, whereas others believe that depression and anxiety might be different presentations of the same general disorder (Dobson, 1985; Watson, 2005).

Mood and anxiety disorders might be difficult to distinguish in part because of the similar way in which stress to bodily systems affects both disorders and the affective states seen in individuals suffering from these disorders (Charmandari et al., 2005; Watson, 2005). Psychopharmacological and psychotherapeutic interventions for mood and anxiety disorders take advantage of this close relationship. Selective serotonin reuptake inhibitors (SSRIs) and cognitive and behavioral therapies both have strong evidence for efficacy in the treatment of these disorders, suggesting similar mechanisms for the etiology of both. Mood and anxiety disorders also are commonly comorbid with other disorders such as substance use disorders (Kessler et al., 2007; Mineka et al. 1998; Watson, 2005).

Because of the high prevalence of depression and anxiety among military members leaving OEF/OIF, the intent of the present study was to model stress-related outcomes observed in military combat veterans by including measures designed to detect anxiety-like and depression-like responses. The experiments used an animal model to measure responses to stress that have been shown to be consistent with anxiety and depression.

Substance Abuse

Stress, mood, and anxiety disorders are increasingly linked with substance abuse (Goeders, 2004). There are several hypothesized explanations for the link between stress and substance abuse, including the self-medication hypothesis (Khantzian, 1985), the tension-reduction hypothesis (Conger, 1956), and stress response dampening (Sher, 1987). There is also research which

suggests that the relationship between substance and stress is mediated by genetic factors or other individual biological and environmental differences (Grunberg, et al., in press). Each of these hypotheses explains substance use in terms of its ability to suppress tension associated with stressors, relieving anxiety, irritability, and depression for the substance user. In general, people exposed to stressors such as unhappy intimate relationships, poor job satisfaction, or harassment, report above average rates of substance abuse (Goeders, 2004).

Of particular interest to this investigation of military-relevant stress research is alcohol use, partly because of the high prevalence of alcohol use in relation to other mental health disorders, and partly because it is legal and readily obtainable. Hoge and colleagues (2004) reported that between 24% and 35% of returning personnel indicated that they had recently used alcohol in excess. A more recent post-deployment reassessment indicates that over 10% of redeployed personnel admitted alcohol problems, such as drinking more than they intended, at six months post deployment (Milliken, et al., 2007). These data indicate that alcohol consumption might be related to stress responses for some period after the initial stressor has ceased.

Various strains of rat voluntarily consume alcohol, allowing the manipulation of stressful conditions to determine how stressors affect alcohol consumption or how alcohol consumption alters responses to stressful conditions (Boulouard et al., 2002; Chester et al., 2004; Darbra et al., 2002; Henniger et al., 2002; Gallate & McGregor, 1999; Le et al., 2001; Linseman, 1987; Pohorecky,

2006). Stressors such housing, immobilization, hierarchical status, and depression-like behavioral markers are related to increased alcohol intake in rats (Chester et al., 2004; Overstreet et al., 2006; Pohorecky, 2006; Wolffgramm & Heyne, 1995). There also is strong support for genetic determinants of alcohol consumption in rats, with differences existing based on genetic strain, and genetic lines selectively bred for anxiety and alcohol preference (Baigent, 2005; Chester et al., 2004; Henniger et al., 2002; Le, 2001; Linesman, 1987).

Individual Differences in Stress Effects and Responses

Empirical research suggests that the effects of stress, particularly mood disorders, anxiety disorders, and substance abuse, might be affected by individual traits including sex, genetics, or age. Anxiety and mood disorders can be induced by the stress response and might differ based on individual factors. Because the United States military is as diverse as it has been at any time in history, military-related research must consider the role of genetic differences, gender, age, ethnic, and other underlying genetic differences. The current research attempts to begin to meet this goal by including different genetic strains, sex, and age in animal subjects.

Genetics

There is compelling evidence that anxiety and mood disorders are determined both by genetic components and by their ultimate phenotypic (e.g., behavioral, morphologic) expression determined by environmental factors (Leonardo & Hen, 2006). Twin studies have attributed approximately 30 – 40% of variance in the incidence of anxiety and mood disorders to genetic variation

(Hettema et al., 2001b; Sullivan et al., 2000). Levels of anxiety tend to persist over a lifetime with little fluctuation, reflecting a potential difference in brain structure or function between highly anxious and less anxious individuals (Kagan & Snidman, 1999; Leonardo & Hen, 2006; Schwartz et al., 1999). Differences in the brains of high and low anxiety individuals might be the result of differences in genetic makeup as well as environmental factors.

As discussed in preceding sections, anxiety and mood disorders are highly comorbid, occurring together in nearly 60% of cases (Kessler et al., 2007). The tendency for anxiety and depression to coexist in families with high incidence of each condition suggests similar etiologies for anxiety and depressive disorders (Ninan & Berger, 2001). Study of the genetic variance contributing to disorders is complicated because it is possible that genetic factors affect not only risk for a disorder, but also how individuals interact with the environment (Leonardo & Hen, 2006). This distinction becomes important when one considers that genetic predisposition accounts for 30 – 35% of the risk for developing PTSD following a traumatic event (Goldberg et al., 1990; True et al., 1993). In addition, there is a genetic predisposition to specific types of trauma, with genetics accountable for 20% of the risk for adverse exposure to assault and 35% of the risk associated with exposure to combat trauma (Lyons et al., 1993; Stein et al., 2002). The existing literature on genetic determinants in response to stressful events is relevant to military populations.

Research using rodents has yielded different responses to stress based on strain differences. Bielajew and Merali (2002) suggested that exposure to

chronic periods of mild stress significantly attenuated corticosterone levels in Long-Evans (LE) rats but not Sprague-Dawley (SD) rats after acute stress exposure. That is, given a history of exposure to non-acute, protracted periods of mild stress and followed by exposure to an acute stressor, corticosterone levels in LE rats were significantly below controls and SD rats, suggesting a blunted stress effect on LE rats. Faraday (2002) reported strain differences in response to daily restraint stress, with SD rats displaying increased acoustic startle response when compared to LE rats in similar experimental conditions. Faraday (2002) also reported strain and sex interactions in open-field activity and pre-pulse inhibition of the acoustic startle response, with only SD females displaying depression-like behavior in the open field and only LE females exhibiting reduced pre-pulse inhibition.

The research presented on genetic differences indicates that there is differential vulnerability and response to stressors based on underlying genetic characteristics. The proposed research includes two strains of rats (SD and LE) to address genetic differences relevant to stress responses. Phenotype— the observable characteristics of an organism resulting from interaction of underlying genetics and environmental factors—has been associated with variations in response to experimental manipulation in rats, as has been noted previously in this paper.

Gender/Sex

It has been recognized for many years that men and women are at differential risk for numerous illnesses and disorders (Baum & Grunberg, 1991).

Considering a complex assortment of psychological, social, and psychobiological variables, differences between men and women can be attributed to any of a number of dimensions including: differences in appraisal, responses, relationships, substance use, and work habits (Baum & Grunberg, 1991). Recent data from the National Comorbidity Survey of 12 month DSM-IV disorder prevalence in the United States indicate that women are diagnosed with anxiety disorders and mood disorders at a higher rate than are men (Kessler et al., 2005). Specific disorders in which women outnumber men include Generalized Anxiety Disorder (GAD), Posttraumatic Stress Disorder (PTSD), and Major Depressive Disorder (MDD). Men are reported to have a higher prevalence of alcohol and substance use disorders than women (Kessler et al., 2005). In a study of primary health care centers from 15 countries around the world, Gater and colleagues (1998) reported that women were diagnosed with depression at higher raters in each center, with an overall odds ratio of 1.60. In terms of anxiety disorders, specifically GAD, there was more variance, but women were more like to be diagnosed with anxiety than men. These results suggest that in the case of anxiety, differences in social roles and experiences might contribute more variance to anxiety disorders than to depressive disorders (Gater et al., 1998). Taylor, Klein, Lewis, and colleagues (2000) characterized the stress response in two distinct ways, depending on sex. Taylor et al. propose that males exhibit a more classic “fight-or-flight” response to stressors, whereas females are more likely to “tend-and-befriend” or, in other words, nurture and engage in behaviors intended to promote safety and decrease distress.

Different stress responses also exist in the animal research literature. In a study of effects of stress during the adolescent period on responses during adulthood, Pohl et al. (2007) reported that depression-like responses were observed in female, but not male rats, whereas anxiety like-responses were observed in male and female rats. Additionally, a line of research investigating the stressful effects of withdrawal from nicotine reported that severity of withdrawal (a stressor) differentially affected animals based on the sex, strain, and age of animals (Hamilton, 2008; Perry, 2007).

The weight of the empirical evidence indicating gender and sex differences in stress responses, combined with the fact that the proportion of women has increased to over 16% of the military force (GAO, 2005), provides clear rationale for including sex as a variable in the present work. Differences in the type, intensity, and duration of stressors and their effect on stress responses based on sexual differences provide valuable information as more personnel are exposed to increasingly taxing situations.

Individual Stress History

Exposure to stressful events early in life can have profound effects on subsequent development and vulnerability to mental health problems (McEwen, 2003). A follow-up of children who survived a bus/train collision revealed that those who were on the bus exhibited more symptoms of depression, post-traumatic stress, and other indicators of maladjustment seven years later than did children on other buses who might have witnessed the accident (Tyano et al., 1996). This effect was persistent even after accounting for other negative life

events since the accident. Vinokur and colleagues (1987) reported that the additive effects of exposure to adolescent stressors, prewar stressors, and war stressors produce long-lasting, adverse effects on mental health that are evident in unemployed Vietnam veterans. Exposure to war was based on respondents having received incoming enemy fire and encountering mines and booby-traps. Mental health in this study was assessed using an index composed of subscales to screen for depression, anxiety, resentment, low self-esteem, and low satisfaction with life (Vinokur et al., 1987).

Key Experiments Relevant to the Present Research

Experiments using animal subjects provide support for age-dependent stress on subsequent behaviors relevant to health. Rodent research has shown behavioral, structural, and chemical changes resulting from early stress exposure (Andersen & Teicher, 2004; Cohen et al., 2007; Lepore & Bobinchock, 2003; Pohl et al., 2007; Romeo et al., 2006; Romeo & McEwen, 2006). Cohen et al. (2007) exposed male Wistar adolescent rats to “potentially traumatic experiences” (PTEs), specifically predator (cat) odor and placement on a platform located above a pool of water. Exposure to PTEs during youth had significant and lasting effects on anxiety-like behavioral responses during adulthood. The measures included acoustic startle response, elevated plus maze behavior, and heart rate variability. Cohen and colleagues (2007) also reported that stress-exposed animals were predisposed to anxiety-like behavior upon re-exposure to stress during adulthood. However, Cohen et al., studied only male

animals of a single strain and did not include a measure of depression-like behavior or alcohol consumption.

Pohl and colleagues (2007) used two stress paradigms, chronic mild stress and severe sporadic stress, to examine the effects of these stressors during adolescence in male and female Long-Evans rats on measures of depression- and anxiety-like behavior in adulthood. Chronic stressors included several hours each of: strobe light exposure, 40 degree cage tilt, white noise, water deprivation, and overnight illumination. Sporadic severe stress consisted of water immersion combined with restraint stress and foot shocks. Although there were sex differences, all animals displayed adverse effects on measures of mental health including: probe burying (index of anxiety and depression), escape behavior (index of anxiety), decreased sucrose consumption (index of depression), and transfer of food preference (index of anxiety and depression) as adults, based on the stressors during adolescence. These findings are interesting and relevant to the present work. However, this study investigated only one strain of rat and used sucrose consumption as a model for depression rather than the Porsolt (1977) forced swim paradigm, which is considered the gold-standard for modeling depression-like behavior in animals. Also, alcohol consumption was not used as a dependent variable in Pohl et al.'s study.

Several researchers report changes in brain structure such as hippocampal development (Andersen & Teicher, 2004) as well as neurochemical changes stemming from stress-induced alterations of the HPA axis (Romeo &

McEwen, 2006; Romeo et al., 2006). Andersen and colleagues (2004) suggest that stress during adolescence results in changes in adult brain structure in rats.

Understanding the effect of exposure to stressors and resulting stress responses is relevant to military populations. Understanding of stress responses and the effect of individual differences is particularly relevant to military personnel as they transition from unpredictable and austere conditions to normal life at home. The current research examined immediate and longer-term effects of stressors that are likely to be relevant to the stress experienced by military personnel.

The Value of an Animal Model

The present research examined the effects of different types of stress during late adolescence on indices of depression, anxiety, and alcohol use during adulthood. This research used a rodent model to prospectively study the effects of adolescent stress on behaviors relevant to health for several reasons. First, an animal model allows for a controlled environment in which causation between the stressor and behavioral responses can be determined, controlling for confounding variables inherent to the human experience. Second, the shorter life span and brief duration of life stages in rats relative to humans allows for the manipulation of stress and the results of the manipulation to be observed through the animals' adolescent and adult phases of development within several months—a study that could take many years in humans. The rat life span is approximately 2 years according to Charles River Laboratories (Parady, personal communication, April 6, 2009). Compared to human life expectancy, which was

reported as approximately 78 years overall (NCHS, 2009), rat life span is 1/39 that of humans. Third, study of rodents allows for control and measurement of behavioral measures such as indices of anxiety- and depression-like behavior, food consumption, water consumption, and voluntary alcohol consumption, as well as biological measures such as body weight and blood collection for corticosterone (a stress hormone) measurement. Predator stress in an animal model, manipulated by predator odor, provides a behavioral and biological model of a life-threatening stressor (Takahashi et al., 2005; Morrow et al., 2000) which elicits responses and behaviors similar to human stress responses. For example, military personnel in specialized training exposed to unpredictable, potentially threatening stress, exhibited increases in blood cortisol concentrations (Morgan et al., 2001) in a manner similar to the corticosterone increases observed in the animal stress literature (Morrow et al., 2000). Predator stress (with accompanied unpredictable stimuli) and sleep disruption was used in the present research because they both model actual human circumstances in deployed settings. Measures such as body weight, food consumption, water consumption, and alcohol consumption provide face-valid measures relevant to humans.

The present research utilized sleep disruption as a stressor, although there are few studies investigating sleep in OIF/OEF. There are, however, anecdotal accounts provided by soldiers through several media outlets and personal communications (Brown, personal communication, September 2007; Johnson, personal communication, October, 2007). Many combat units allow 4

hours of sleep during each 24 hour period, and many individuals are unable to obtain even this short period of rest because of sleep disturbance related to noise, sleep disorders, or extended mission requirements (Morin & Hu, 2007). The present research included sleep disruption in animals, allowing the manipulation of a potentially debilitating stressor. Similar to humans, rats are susceptible to the deleterious effects of environmental noise on sleep (USACHPPM, 1995; Kawanda & Suzuki, 1999; Rabat, 2004, 2005, 2006).

Research in humans has shown differential responses to stress based on genetic variation (Hettema et al., 2001a; Sullivan et al., 2000), and rodent models have shown differences in stress responses based on sex and strain differences (Bielajew & Merali, 2002; Faraday, 2002). Biological and behavioral differences in stress responses observed in humans and animals suggest that rodents are a valid model for the human stress condition.

Overview of Research

The present research examined effects of stress during late adolescence on subsequent behaviors relevant to health during adulthood in male and female Sprague-Dawley (SD) and Long-Evans (LE) rats. The specific behaviors analyzed are behavioral indices of anxiety (time spent in the center of an open-field chamber), depression (immobility when forced to swim in an inescapable cylinder of water), and voluntary consumption of ethanol.

This research project was conducted in two experiments. Experiment 1 investigated the effects of predator stress and sleep disruption in adolescent male rats. Experiment 2 expanded the design of Experiment 1 by examining the

effects of predator stress and sleep disruption on male and female rats of two strains, Sprague-Dawley and Long-Evans. The experimental protocol was approved by the USUHS Institutional Animal Care and Use Committee (IACUC) and was conducted in full compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (National Institutes of Health [NIH], 1996).

Specific Aims

The specific aims of the research were:

(1) To determine how predator stress during adolescence affects behavioral indices of anxiety, depression, and alcohol consumption during adulthood;

(2) To evaluate how sleep disruption during adolescence affects behavioral indices of anxiety, depression, and alcohol consumption during adulthood;

(3) To evaluate the combined effects of predator stress and sleep disruption during adolescence on behavioral indices of anxiety, depression, and alcohol consumption during adulthood;

(4) To evaluate effects of stress during adolescence on behavioral indices of anxiety, depression, and alcohol consumption during adulthood in male and female rats of two different strains (genotypes).

Relevance of an animal model in the proposed research

Rats were chosen as subjects because they provide a valid and reliable model to study involving the effects of stress. The genetic similarities between rats and humans are striking, with many researchers believing that each have similar numbers, 90% of which are shared (Gibbs et al., 2004; Stein, 2004). As

such, the literature reports use of rats in animal models of myriad human conditions including drug abuse, stress, and mental and physical health disorders (Acri, 1994; Elliott et al., 2004; Elliott et al., 2003; Faraday, 2000, 2002; Winders & Grunberg, 1989).

With regard to the present research, for example, rat models have been used extensively to investigate actions of nicotine, stress, environmental conditions, and alcohol for over 40 years (e.g., Acri et al., 1994; Balfour et al., 1986; Barron, et al., 2005; Benwell & Balfour, 1985; Glick et al., 1970; Goldberg et al., 1981; Marks et al., 1986; Corrigall & Coen, 1989, 1991; Hansen et al., 1979; Slotkin et al., 1986). Further, rats are most commonly used to study effects of environment on actions of drugs of abuse (Bowling, Rowlett, & Bardo, 1993; Bowling & Bardo, 1994; Boyle, Gill, Smith & Amit, 1991; Phillips, Howes, Whitelaw, Wilkinson, Robbins, & Everitt, 1994). Our laboratory has used rats, mice, other rodents, and primates in research over the past 25+ years to model and predict human responses. Findings from our laboratory with rats have been reliable (in our laboratory and in other laboratories) and have predicted effects in human subjects and human populations with regard to stress, body weight, and drug actions.

With regard to genetically based differences in the current work, two strains were utilized to observe differential responses based on phenotypic characteristics--specifically coat, skin, and eye color. Of the over 20,000 genes in the rat, five or fewer genes are thought to be central to eye and coat color differences between strains (NBII, 2008; McCubbins, 1963). Interestingly, of the

20,000 - 25,000 genes in humans, less than five genes are thought to play a meaningful role in skin and eye color between different people (Cheng et al., 2005; Rebbeck et al., 2002). Although both rats and humans are more genetically similar to their same-species counterparts than they are different, there remain differences observed based on seemingly simple phenotypic characteristics. Given the close genetic relationship with humans and the differential biobehavioral responses observed in both rats and humans based on phenotype, rats are considered to be a good model for this work.

Independent Variables

The independent variable in Experiment 1 was treatment condition. In addition to treatment condition, Experiment 2 included sex and strain. In Experiment 2, the subjects of interest were male and female, Sprague-Dawley (SD) and Long-Evans (LE) rats. Animals in both experiments were exposed to four possible stress conditions: no stress, predator stress, sleep disruption, predator plus sleep disruption (combined). The following paragraphs describe each independent variable. More detailed explanations of the specific variables follow in a later section.

Sex

Male and female rats were used (in Experiment 2) for several reasons. First, this research was designed to model human experiences, and sex differences are germane to the human condition. Second, sex differences in response to stressors have been observed in human and animal literature, with biological and behavioral differences observed between male and female rats

(Baum & Grunberg, 1991; Faraday, 2000; Kessler et al., 2005; Klein et al., 1996; Taylor, Klein et al., 2000). Sex is among the most fundamental of individual characteristics and, therefore, it is included as a variable of study in the current work.

Predator Stress

Predator stress is a painless, acute stressor that has proven effective in rodent models of stress exposure. The present research used a predator stress paradigm that involved exposure to fox (a natural predator of rats) urine combined with other environmental stimuli. Odors emitted by predators such as foxes, cats, and ferrets (including feces and urine), are biologically relevant because they induce an innate biochemical stress response in rodents (Takahashi et al., 2005; Day et al., 2004; Campbell et al., 2003; Hayley et al., 2001b; Morrow et al., 2000; Funk & Amir, 2000). Predator stress exposure produces behavior changes in rodents that include: food consumption changes, altered startle response, altered locomotion, and exploratory behavior (Adamec et al., 2006; Belzung et al., 2001; Endres et al., 2005; Masini et al., 2005; Korte et al., 2005). The added environmental stimuli, including unpredictable noise (e.g., alarm, whistle), bright lights, and periodic cage shaking, were administered on a variable basis to enhance the unpredictability of the novel environment in each day of stress manipulation. Fox urine was used in these studies because it is readily available on the retail market, relatively simple to administer, and it produces robust and significant stress effects in experiments conducted with rats in our laboratory (Berger, personal communication, 2007). This research utilized

predator stress to model an acute, unpredictable stressor, such as that which would be experienced by soldiers fearing imminent attack.

Sleep disturbance

Disrupted sleep and sleep deprivation create distress in animals and humans (Kavanaugh, 2005; Rabat, 2004; Rechtchaffen & Bergmann, 2002; Belenky, 1997). Methods of depriving or disturbing sleep in rats in previous studies have included total sleep deprivation (Everson et al., 1989) and exposure to environmental noise (Rabat et al., 2006, 2005, 2004). Total sleep deprivation results in lesions of the paws and tails, loss of weight despite increased food intake, and death or impending signs of death within 2 – 4 weeks (Everson et al., 1989). Rabat et al. (2006, 2005) reported that chronic exposure to environmental noise resulted in cognitive deficits, permanently disrupted circadian rhythm, disruption of slow-wave (deep) sleep, disruption of paradoxical (REM), and increased locomotor reactivity. The present experiment used variable high and low frequency environmental sounds (banging, bells, voices, shattering glass, etc.) to disrupt sleep intermittently throughout the sleep cycle in rats and to model deleterious sleep environments encountered by humans. The present research used sleep disruption (but not total deprivation) as a stress condition because it has importance in the human condition and to model military operational conditions.

Dependent Variables

The key dependent variables measured in this work were open-field activity, immobilization during a forced swim procedure, ethanol consumption,

and serum corticosterone levels. The current section provides a brief description of each dependent variable, followed by detailed descriptions of the exact procedures and equipment used.

Open-Field Locomotion

Locomotor activity is a collection of unconditioned ambulatory behaviors in a particular environment. Rodent movement in a novel open-field has been used to measure effects of experimental manipulations which include stress effects, exploration, and general movement (Campbell et al., 2003; Boguszewski & Zagrodzka, 2002; Elliott & Grunberg, 2005; Faraday, 2000; Grunberg et al., 1984; Pare et al., 1999). The open-field paradigm is based on a rodent's instinctive tendency to move along the perimeter of a novel environment when it senses threat. The duration of time in the center of the open field is considered to be inversely related to anxiety levels. In the present experiment, a key domain of interest is center time because it can be used as an index of anxiety. It is generally hypothesized that time spent in the center of the open arena indicates less anxiety than time spent at the walls of the arena (Beck & Luine, 2002; Lee et al., 1986). Animals spending more time along the walls of the chamber are hypothesized to be more anxious because the walls provide a sort of protection versus the vulnerability of open area. If animals spend more time in the center, then they are thought to be less anxious because there is no such protective cover in the center of the arena. As a result of these various indices, open field activity provides a useful way to examine effects of experimental manipulations on stress and its effects on anxiety-like behavior. Locomotion in the current

work, particularly center time, was used as an indicator of anxiety and overall movement provided a measure of general health of the subjects. Open field activity was measured at three points during the course of the experiment: prior to stress exposure, immediately after the final day of stress exposure, and two weeks after the last day of stress.

Forced Swim Test

Seligman established learned helplessness as the gold standard for modeling depression (1968, 1974). The Forced Swim Test (FST) has been widely used as a model of learned helplessness or depression in rodents (Petit-Demouliere et al., 2005; Carlezon et al., 2002; Pliakas, 2001; Detke et al., 1995; Porsolt et al., 1977). The FST is based on the observation that rats forced to swim in an inescapable container have an initial period of activity, eventually moving only to the extent required to keep their head above water (Porsolt, 1977). The FST procedure occurs over at least two days and requires two exposures to water: 15 minutes on the first day and 5 minutes on the second day. The proportion of immobility to mobility on day two is considered a measure of the extent of learned helplessness in the animals exposed to the FST. Porsolt et al. (1977) reported that rats in the FST procedure remained immobile for 75% of the administration time, but antidepressant medications reduced immobility and increased escape behavior. The results of Posolt's findings have been replicated in more recent work, reporting effects of antidepressant medications, serotonin, and other treatments (Dableh et al., 2005; Xu et al., 2005; Reneric & Lucki, 1998). The present research used the forced swim procedure to

determine if stressors in adolescence have a causal influence on depressive symptoms during adulthood. The terms “depression-like” or “depressive-like,” rather than “depression” will be used in this work to refer to animal behavior. Because the term depression characterizes an affective component that cannot be assessed in animals, “depression-like” was a more accurate characterization of the behaviors observed.

Alcohol Consumption

Conger (1956) originally proposed the tension reduction hypothesis, which posited that individuals with relatively high anxiety might be more sensitive to alcohol’s anxiolytic effects. Conger’s hypothesis predicts that more anxious individuals will experience more of a reduction in anxiety after consumption of alcohol than will nonanxious individuals and, therefore, will consume more alcohol than nonanxious individuals. Conger’s hypothesis has been debated because of the difficulty of assessing the circumstances which lead to increased alcohol drinking behavior in human beings. Young et al. (1990) proposed a revised tension reduction hypothesis based on the variability in tension reduction based on expectancies of alcohol’s tension-reducing effects, and interactions of situational, biological, and gender-related factors.

Oral self administration of alcohol by rodents provides a face-valid paradigm to model human alcohol consumption. Voluntary oral consumption in rats can be established with prior food or water deprivation, by adding sweeteners to the alcohol solution, or by providing progressively higher concentrations of alcohol, beginning with a low concentration (Gallate &

McGregor, 1999; Wolfgramme & Heyne, 1995). Rats selectively bred for high or low alcohol consumption and for high anxiety consume alcohol at higher rates than do rats not bred for such purposes (Chester et al., 2004; Henniger et al., 2002). The current work used ethanol self-administration in normal rodents (not bred for a specific trait) with progressive ethanol concentrations to examine the extent to which stress affects tendency to consume alcohol.

Blood Corticosterone

The hypothalamic-pituitary-adrenal (HPA) axis is activated in response to a stressor. HPA activity is reflected by blood concentrations of several biochemicals, including corticosterone (CORT) (Belz et al., 2003; Hennessy, 1997; Pham et al., 1999b; Selye, 1973). Stress creates consistent changes in corticosterone levels in animals. Investigations that examine biological markers of stress routinely examine levels of plasma corticosterone (Brown & Grunberg, 1995; Faraday, 2002; Belz et al., 2003). Restraint stress, in particular, has been routinely used to produce increases in corticosterone (Acri, 1994; Kant et al., 1987; Raygada et al., 1992). A study by Berger (personal communication, 2007) revealed robust corticosterone effects when exposing rats to fox urine in a predator stress paradigm. The current experiment examined the extent to which stress during adolescence affects corticosterone concentrations. Following completion of the study, subjects were anesthetized by carbon dioxide inhalation following current Center for Laboratory Animal Medicine (LAM) practices, and decapitated with a rat guillotine to collect trunk blood for serum corticosterone assay. Serum corticosterone was assayed by an ImmunoChem Double-Antibody

radioimmunoassay (RIA) kit using ^{125} I-labeled corticosterone (ICN Biomedicals, Costa Mesa, CA). A limited amount of specific antibody is reacted with a fixed quantity of ^{125} I-labeled corticosterone. The concentration of unlabeled corticosterone in samples increased as a function of the decreasing percentages of bound radioisotope-labeled corticosterone. A second antibody precipitates antibody bound to antigen. The quantity of endogenous corticosterone was determined by measuring the radioactivity of the precipitate with known standards from the same assay in a gamma counter and converting disintegrations per minute (DPM) into concentrations. All samples and standards were run in duplicate. The sensitivity of the assay is 8 ng/ml (Faraday, 2000) and the coefficient of variation is 6.93%. This measure was included to verify that predator or sleep stress are indeed stressors (as assessed by HPA axis activity).

Preliminary Studies and Relevant Laboratory Experience

All techniques required for the proposed research were developed and are available in the Grunberg laboratory. In addition, the investigator had experience in designing, conducting, and analyzing data from similar experiments. The scientists involved in assisting with this work were highly experienced in conducting the measures utilized.

The investigator's master's research examined behavioral effects of nicotine withdrawal in adolescent male and female rats of two strains, Sprague-Dawley (SD) and Long-Evans (LE) (Perry, 2007). Relevant to the present research, this experiment included the measurement of open field activity and other behaviors relevant to health. The investigator designed, planned, and

managed each element of this project, analyzed the data, and wrote up the findings. This project involved many independent and dependent variables relevant to the present project.

The investigator also assisted with eight other research projects during which he obtained experience in measures, including water and alcohol consumption, food consumption, and social interaction (Simpson-Mckenzie, 2008; Tomchesson, 2006). The alcohol consumption experiment informed the modified technique used in the proposed project.

The laboratory personnel are well versed and skilled in measuring every variable utilized in the present research. The expertise of the lab includes assessment of biochemical measures. Corticosterone assays are a routine procedure within the laboratory (Faraday, 2000, 2002; Faraday et al., 2005; Berger, personal communication, 2007).

Recently, the laboratory has extended its expertise by adding several new measures relevant to the present research. In particular, the Porsolt forced swim paradigm and the predator stress, fox urine paradigms were developed and both techniques were used successfully in Experiment 1

EXPERIMENT 1

Overview

The purpose of Experiment 1 was to examine the effects of stressors during adolescence in male Sprague-Dawley (SD) rats on subsequent measures of mental health during adulthood. Sprague-Dawley albino rats are the most commonly used laboratory rats. The stress conditions in Experiment 1 were: no

stress, predator stress, sleep disruption, and predator plus sleep disruption (combined). Measurements were taken before, during, and after stress. The experiment lasted seven weeks. The dependent variables were open-field locomotion, forced swim immobility, ethanol consumption, and blood corticosterone.

Hypotheses

There were four major hypotheses based on four dependent variables: (1) center time (a measure of anxiety-like behavior); (2) forced swim test performance (a measure of depression-like behavior); (3) ethanol consumption; and (4) serum corticosterone levels. **Hypothesis 1: Serum Corticosterone**

It was hypothesized that animals in stress conditions would display elevated serum corticosterone when compared to non-stressed rats. Past research has indicated elevations in serum corticosterone levels in stressed rats (Acri, 1994; Kant et al., 1987). A study conducted by Berger using fox urine as a predator stressor also produced significant corticosterone increases in rats (personal communication, 2007). Hairston et al. (2001) also reported significant increases in corticosterone in rats deprived of sleep. It was predicted that both predator stress and sleep disruption would have similar effects on corticosterone levels, with the combined condition resulting in cumulative stress effects. (Predicted direction for corticosterone: Combined > Predator = Sleep > Control)

Hypothesis 2: Open Field Locomotion (Center Time)

It was hypothesized that stressed animals would exhibit different locomotor behavior when compared to non-stressed animals. Rats in the

predator stress condition were expected to spend less time in the center of the open field than unstressed rats. Male rats in the sleep disruption condition also were expected to spend less time in the center of the open field, indicating greater anxiety. This hypothesis was based on work by Faraday (2002) and also Pohl et al. (2007), who reported that young male rats exposed to sporadic severe stress exhibited anxiety responses at a rate higher than that of non-stressed rats. Animals in the combined condition were expected to have less center time that either predator stress or sleep disruption. (Predicted direction for center time: Control > Sleep = Predator > Combined at all phases after stress)

Hypothesis 3: Forced Swim Test Performance

It was hypothesized that rats in the predator stress and sleep disruption conditions would display greater immobility and swim less during the forced swim procedure than non-stressed rats. Based on Pohl et al. (2007), it was expected that stress exposure would result in relatively greater depression-like behaviors (more immobility and less swimming) for males exposed to stressors. (Predicted direction for immobility: Combined > Predator = Sleep > Control at all phases after stress)

Hypothesis 4: Alcohol Consumption

It was hypothesized that animals in the predator stress condition would consume more ethanol than animals in the sleep disruption condition. Animals in the predator stress and sleep disruption conditions were expected to drink more ethanol than non-stressed rats. This hypothesis was based on Pohl (2007) in which different types of stress have differential effects in male rats. Specifically,

Pohl (2007) reported that male rats were more responsive to severe, sporadic stressors than to mild chronic stressors. In the current work, repeated episodes of predator stress were expected to be analogous to severe, sporadic stressors. Animals in the combined condition were expected to display increased ethanol consumption as a result of the cumulative stress effects of predator stress and sleep disruption. (Predicted direction for alcohol intake: Combined > Predator > Sleep > Control)

Methods

Experimental Design and Sample Size

Sample size was determined in two ways: (1) based on previous experiments, and (2) using procedures outlined by Keppel (1991) and Cohen (2003). The sample size (cell size of n = 10) was determined (1) based on previous reports using similar dependent measures and responses to various stressors (e.g., predator scent stress, restraint stress, water emersion, and elevated platform) (e.g., Cohen et al., 2007; Pohl et al., 2007; Morrow et al., 2000), and (2) a power analysis based on previous research using stress as an independent variable.

Studies in the research literature report statistically significant effects using cell sizes of between 6 – 16 animals for crowding, restraint, predator scent, and platform stress effects (e.g., Brown and Grunberg, 1995; Cohen et al., 2007; Day et al., 2004; Faraday et al., 2003; Funk & Amir, 2000; Morrow et al., 2000) and 4 – 7 animals for sleep disruption or deprivation (Rabat et al., 2005). Studies utilizing the forced swim test as a model of anxiety report statistically significant

effects using samples sizes of 8 – 12 animals per cell (Kirby & Lucki, 1997; Xu et al., 2005). Ten subjects per cell were used in this project as a conservative sample size to achieve adequate power with effects for predator stress, sleep disruption, forced swim immobility, and biological measures.

The sample size and power for the proposed research also was determined with an automated computer program (GPower, version 3.0.3) to enhance efficiency and precision (Erdfelder et al., 2006). The results of stress effects on swim immobility reported by Shalyapina et al. (2007) yielded a large effect size of 1.5 with a sample size of 12 animals per cell. Using 10 animals per cell in the current experiment and an effect size of 1.5, the power for swim test immobility was predicted to be .94. The results of the swim immobility test administered by Shalyapina et al. (2007) yielded a large effect size of 1.49 with a sample size of 12 animals per cell. Using 10 animals per cell in the current experiment and an effect size of 1.49, the power for swim test immobility was expected to be .94. The results of the stress on alcohol consumption reported by Porhorecky (2006) yielded a large effect size of 2.7. Using 10 animals per cell in the current experiment and an effect size of 2.7, the power for alcohol was expected to be .99. Measures of locomotor activity and corticosterone are well established in this lab and have shown significant effects and power of at least .80 in sample sizes of 8 subjects or more.

Subjects

The subjects were 40 adolescent male Sprague-Dawley rats (10 per condition) 22 days old at the beginning of the experiment from Charles River

Laboratories. Investigators have defined adolescence in the rat as 21 – 42 days for female rats and 21 – 55 days for male rats (Spear & Brake, 1983; Ojeda and Urbanski, 1994Faraday, Elliott, & Grunberg, 2001). Sprague-Dawley albino rats are the most commonly used laboratory rats, and provide a good model for a variety of human conditions. At the beginning of the experiment, the animals weighed an average of 50 grams.

Animals were pair-housed in standard rat cages (42.5 x 20.5x 20 cm) on hardwood chip bedding (Pine-Dri) with continuous access to food (Harland Teklad 18% Protein Rodent Diet 2018 pellets) and water. Pair housing was used, because rats are social animals. Individual housing and isolation can elicit stress-like changes in behavior and physiology of rats, including emotional reactivity and cardiovascular function (Brown & Grunberg, 1995; Lawlor, 2002). Pair housing also modeled the social environment experienced by military forces. The animals were housed in two separate rooms divided by the following conditions: Room 1 – no stress group and predator stress group; Room 2— sleep stress group and combined sleep plus predator stress group. Two housing rooms were required for the sleep disruption condition, because sound exposure was utilized to disrupt sleep in one of the rooms while animals in the no stress condition were in a relatively noise-free environment. Housing rooms were maintained at 67 – 70 degrees and about 60% humidity on a 12:12 hour reversed light-dark cycle in order to match the nocturnal rats' waking and active period with the hours most ideal for observation.

Independent Variables

Predator Stress

The steps for the predator stress procedure were established by Berger (personal communication, 2007) and have elicited increased biochemical indicators of stress response. During predator stress exposure, the animals were transferred from their home cages and housing room to Plexiglas lid-covered "stress cages" located in a procedure room separate from the housing. The stress procedure lasted 10 minutes and occurred at unpredictable periods during the active phase of the light cycle. Fox urine (15mL, Buck Stop Lure Co., Inc., Stanton, MI) was placed on a large cotton balls and placed in varying spots in each stress cage. A bright florescent overhead light remained on. On day 1, only the fox urine was presented. On days 2-14, additional stressors (e.g., additional bright light, noises, or cage shaking) were combined with the fox urine (see Table 1 for specific schedule of stressors). Noises included the dinging of a lab timer at the 3, 5, and 8 minute mark during the fox urine exposure, several blows of a standard police whistle at the 2, 6, and 8 minute marks during the urine exposure, single blows of a standard whistle at the 2 and 6 minutes marks during the fox urine exposure, shaking of coins in a metal container at the 3, 6, and 8 minute marks during the exposure, or flashing the overhead florescent lights at various points during the other stress exposures.

Sleep disruption

Half of the rats in the experiment were exposed to various recorded sounds during their low-activity or sleep period for a period of 14 days. Rats

exposed to sound were housed in a room separated from animals in a quiet environment by a cinderblock wall. Rabat (2007) indicated that varying frequencies of sound as well as unpredictability of patterns and type of noise all contributed to sleep disruption in animals. Therefore, various sounds were recorded on compact discs (CDs) and played on a clock/radio/cd player (Sony Dream Machine, Model # ICF-CD843V) programmed to play on an hourly loop for nine hours of the animals' 12-hour light (sleep) period. Sounds played intermittently for one hour, including periods of silence of varying lengths. The shortest sound played for 6 seconds, and the longest sound played for 1 minute, 10 seconds. The shortest period of silence during each hour was 2 minutes, and the longest period of silence in each hour was approximately 17 minutes. The sound level in the room prior to sound exposure was approximately 59 decibels (dB). Sounds exceeding 85 dB are thought to be harmful to rodents; therefore, recorded sounds ranged from 65 dB to 80 dB. Sound duration and frequency was altered at seven days to adjust for habituation. Total hourly sound exposure did not exceed 6 minutes at any time during the experiment.

Dependent Variables

Serum Corticosterone

Following completion of the study, subjects were anesthetized by carbon dioxide inhalation following current LAM practices, and decapitated with a rat guillotine in order to collect trunk blood for serum corticosterone assay. Serum corticosterone was assayed by an ImmuChem Double-Antibody radioimmunoassay (RIA) kit using ^{125} I-labeled corticosterone (ICN Biomedicals,

Costa Mesa, CA). A limited amount of specific antibody is reacted with a fixed quantity of ^{125} I-labeled corticosterone. The concentration of unlabeled corticosterone in samples increases as a function of the decreasing percentages of bound radioisotope-labeled corticosterone. A second antibody precipitates antibody bound to antigen. The quantity of endogenous corticosterone was determined by measuring the radioactivity of the precipitate with known standards from the same assay in a gamma counter and converting DPM into concentrations. All samples and standards were run in duplicate. The sensitivity of the assay is 8 ng/ml (Faraday, 2000) and the coefficient of variation is 6.93%. This measure was included to provide a biomarker of stress (as assessed by HPA axis activity).

Open Field Activity

The open-field apparatus is a square acrylic chamber with clear sides and a clear ventilated top in which animal activity is monitored and measured. Open-field activity was measured using an AccuScan/Omnitech Electronics Digiscan infrared photocell system (Test box model RXYZCM [16 TAO], AccuScan Instruments, Inc., Columbus, OH) in a dedicated room with cinderblock walls which help minimize external sounds. Animals were placed individually in 40 x 40 x 30 cm clear Plexiglas arenas with ventilated Plexiglas lids placed on top of the arena during measurement. Activity measurements were obtained during the rats' active cycle (dark period) for a 1 hour period in a dark room. Animals were placed individually into one of the sixteen arenas. Locomotion data were automatically gathered and transmitted to a computer via

an Accuscan Model DCM-I-BBU analyzer connected to each arena. A computer loaded with activity monitoring software and connected to the analyzer collected data for each arena. The software measured 21 activity variables, including center time, total distance traveled, horizontal activity, and vertical activity. Chambers were cleaned between subjects with a 35% isopropyl alcohol solution and paper towels. Cleaning occurred after the animals had been removed from the room and prior to the next set of animals being measured. The chambers were thoroughly cleaned and dried before introducing the animals.

Forced Swim Test

The forced swim test was administered at the end of the two-week non-stress period. Rats were placed individually into a cylinder (approximately 65 cm tall and 25 cm diameter cylinder filled to a height of 30 cm with water at room temperature) on the first day for 15 minutes. After 15 minutes of swimming on day 1, the rats were removed from the water, dried with towels and warmed under heat lamps for 15 minutes. On day 2, rats were tested for a maximum of 5 minutes under conditions identical to day 1 to determine proportion of immobilization (rat ceases escape attempts and appears stationary or immobile) to swimming and escape attempts. After 5 minutes in the cylinder, the rats were removed from the water (Carlezon et al., 2002). Greater proportion of immobility to other behaviors was interpreted as increased helplessness or depression-like behavior. All rats were monitored for the duration of the forced swim test and were removed from the water if they showed signs of distress or appeared to be drowning. One animal was removed from the forced swim chamber during day 1

of the test after it ceased swimming and sank below the surface of the water. The animal survived and was included in subsequent trials.

Forced swim test observers were trained by viewing video of animals in the forced swim paradigm and by observing scrub animals in non-test conditions. Observers first viewed video of animals in a forced swim procedure until a clear distinction was made between immobilization, swimming, and escape behaviors. Observers then gathered to rate scrub animals under conditions similar to an actual test. The observers each independently viewed the same animal for 5 minutes, recorded the behaviors, and then compared their ratings at the conclusion of the 5-minute observation period. This procedure was repeated until inter-rater reliability of $\geq$ 90% was achieved—about three iterations.

Alcohol consumption

The alcohol administration procedure was based on techniques by Henniger et al. (2002) in which rats were given 24-hour access to ethanol in three different concentrations (3, 6, or 12%, as developed in our laboratory by Starosciak, personal communication, 2007) for three days at each concentration in 100ml bottles. This method of alcohol consumption utilized a solution of ethanol and water and observed significant differences in intake and preference between male and female rats bred for high anxiety behavior. The current study used rats bred for general use and not specific traits to model effects of stress and responses to stress based in otherwise normal subjects. Animals were given continuous access to the ethanol solution in their home cages for the duration of the alcohol administration period. Continuous access to water in 500

ml bottles also was provided. Alcohol consumption was measured beginning on day 39 of the experiment and was measured for 9 days.

Procedure

Experiment 1 was conducted in four phases: baseline, stress, post-stress, and adult (see Table 1). The baseline phase was the period from day 1 to day 6, during which animals were acclimated to the environment and gentled for ease of handling. The stress phase was the period from day 7 to day 21, during which the stress manipulations (i.e., predator, sleep disruption, combined) were conducted. Only body weight and food consumption were measured during the stress phase. The post-stress phase was the period from day 21 to day 34 when the animals matured to adulthood. The adult phase was the period from day 35 until day 49, the last day of the study.

Behavioral measures to assess anxiety- and depression-like behavior were taken during the post-stress and adult phases. At the conclusion of the experiments, animals were euthanized by LAM personnel (carbon dioxide inhalation euthanasia from a compressed gas cylinder and decapitation) following the completion of the behavioral testing.

Baseline

The baseline phase lasted for six days. The rats were 22 days old upon arrival and were acclimated to the facility for 3 days. During the baseline phase, the rats were randomly assigned to either no stress, sleep disruption only, predator stress only, or sleep plus predator stress conditions (combined). The animals were then pair housed (within condition) and placed into two separate

rooms – Room 1: Sleep disruption-only and Combined predator stress/sleep disruption; Room 2: Control and Predator stress-only. Each rat was handled for 3 - 5 minutes for the first three days after arrival in order to familiarize them with human contact and ease handling during later stages of the experiment. Animals were placed in open-field chambers on the fourth day after arrival for acclimation to the locomotor apparatus, and a baseline measure of open-field activity was obtained on day five. Body weight, food consumption, and water consumption were measured every two days for the duration of the experiment. Ambient sound levels of each housing room were measured on the day the animals arrived to ensure that there was not interference with the sounds that were manipulated during the experiment. Temperature and humidity also were measured on the days that body weights were recorded to ensure a consistent and healthy living environment for the animals.

Stress Phase

The stress phase began on day seven, with rats 28 days old, and was 14 days in duration. The animals assigned to the predator stress condition were removed from their housing rooms and transported to a separate procedure room for the stress procedure. The predator stress-exposed rats were placed in the presence of fox urine and unpredictable stimuli (e.g., noise, light, cage shaking) for 10 minutes each day. Individual cotton balls were each soaked with 15 milliliters of fox urine (Buck Stop Lure Co, Stanton, MI) and placed inside empty Plexiglas cages with plastic, filtered tops. The white overhead light was kept illuminated throughout the entire procedure. For each day of the 14-day stress

manipulation, the cotton balls were placed in different sections of the cages to reduce the likelihood of habituation. Additional novel stressors, such as bells, clapping, flashing lights, and whistles were used in conjunction with the fox urine to create an unpredictable, more stressful environment. Personnel administering predator stress wore dark-colored scrubs, and not their normal white lab coats, when administering predator stress. Wearing different clothing served two purposes: to reduce the possibility of fox urine contaminated clothing affecting animals not assigned to the predator stress condition upon return to the housing rooms and to take advantage of any conditioned responses to clothing of the individuals administering the stress.

Sleep disruption-only rats were exposed to recorded noises (e.g., banging, talking, coins, doors slamming) during their low-activity period on a nightly variable interval schedule. There were eight recorded noises (downloaded from the Internet and recorded on cd) lasting between six seconds and one minute-ten seconds. The ambient noise level in the sleep disruption room, before sound manipulation, was 59 decibels (dB). The sound level of the recorded noises ranged from 65 dB to 80 dB. Total exposure to noise during a given hour was 5 minutes, 46 seconds. Sounds began 30 minutes after the animals' dark period began and played hourly for 10 hours. Total exposure to sound on each night totaled 54 minutes, 6 seconds. Animals in the sleep disturbed condition also were disturbed by laboratory personnel several times each day to interrupt active period napping. Sound levels were measured and verified using a Larson-Davis

Sound Level Meter, Model 2800A, with a microphone placed in the location of the animal cages for measurement.

Post-Stress Phase

The post-stress phase began on day 21, the day after the last stress manipulation, with the rats at 42 days old. Post-stress open field locomotion was performed on day 21 and body weight, food consumption and water consumption were measured every other day. The total length of the post-stress phase was 14 days to allow the rats to mature to adulthood. Rats are considered sexually mature between 42 and 55 days old (Spear & Brake, 1983). At the end of the post-stress phase, the rats were 55 days old.

Adult Phase

The adult phase began on experiment day 35 with measurement of open field activity on the first day of the adult phase and body weight, food consumption, and water consumption on every second day until the end of the experiment. During the adult phase, forced swim immobility and voluntary consumption of ethanol were measured. The forced swim procedure was conducted over a two-day period. Plexiglas cylinders were filled with 30 cm of water allowed to reach room temperature (approximately 22-23 degrees Celsius). On day 1, animals were placed separately into water-filled containers, swam for 15 minutes without assistance, and then were removed from the container, dried with towels, and placed under a heat lamp for approximately 10 minutes. They were then returned to their home cages. On the second day, the rats were returned to the water filled cylinders for 5 minutes. The rats were evaluated

based on three behaviors: immobility, escape, and swimming. Scorers evaluated the rats' behavior every five seconds for five minutes, for a total of 60 evaluation points (see Table 2). Animals were removed from the water and dried as described above. Proportion of immobility to escape behavior and swimming was measured to obtain an index of depression, with greater immobility indicative of greater depression-like behavior.

Beginning on experiment day 38, the rats had continuous access to ethanol in their home cages at three concentrations: experimental days 38-40 - 3%; experimental days 41-43 - 6%; experimental days 44-46 - 12%. Voluntary alcohol consumption was measured on each day of alcohol administration. Access to water remained unrestricted during alcohol administration.

Body weight was measured using electronic balances programmed to average multiple weight measurements within several seconds to account for movement of animals. Food and water consumption were calculated by weighing food and water containers on alternating days and computing change scores to indicate consumption.

The animals were euthanized via carbon dioxide inhalation euthanasia from a compressed gas cylinder and decapitation on experimental day 49. Blood was collected, centrifuged, and stored at -80°C for later serum corticosterone measurement.

Data Analytic Strategy for Experiment 1

Corticosterone (CORT) median values were analyzed using univariate ANOVAS, and Tukey HSD post-hocs were performed where there were significant main effects..

Open-field data (center-time), forced swim immobility, and serum corticosterone were analyzed with separate analyses of variance (ANOVA). Tukey HSD post-hocs were performed when there were significant main effects.

Body weight, food consumption, and water consumption were analyzed using repeated-measures ANOVAs to assess the extent of consumption over time. Body weight , food consumption, and water consumption also were analyzed cross-sectionally at specific time points using separate analyses of variances. Where there were significant differences at baseline, ANCOVAs were used to account for pre-existing variance. Significant main effects and interactions were examined using separate ANOVAs.

Multivariate analyses of variance were used to analyze overall open field activity, because overall open field activity combined several correlated measures such as horizontal movement, vertical movement, rearing, center time, total distance, total movement, etc.

Repeated-measures ANOVAs were used to analyze Ethanol consumption across each day of Ethanol administration. Individual ANOVAs were used to compare total alcohol consumption at each concentration (3%, 6%, and 12%) and overall consumption.

Forced swim test immobility was analyzed by first obtaining a proportion of immobility to swimming and escape behaviors and median values were calculated to determine central tendency as it relates to immobile behavior. Chi square analysis was then used to compare median values of each of the four groups.

The experiment was designed to provide adequate power (0.80) in order to reduce the likelihood of type II error. In addition, only if overall analyses revealed a significant main effect or interaction were subsequent analyses performed. This strategy reduced the number of statistical tests performed (Cohen et al., 2003; Keppel, 1991). All tests were two-tailed with significance determined by $p \leq 0.05$.

Results—Experiment 1

P values in the document text are presented as (< 0.05) for each significant finding, regardless of actual p value. Statistical tables and analyses with complete details of each analysis are presented in Appendix A. Figures and graphs are listed in the document text.

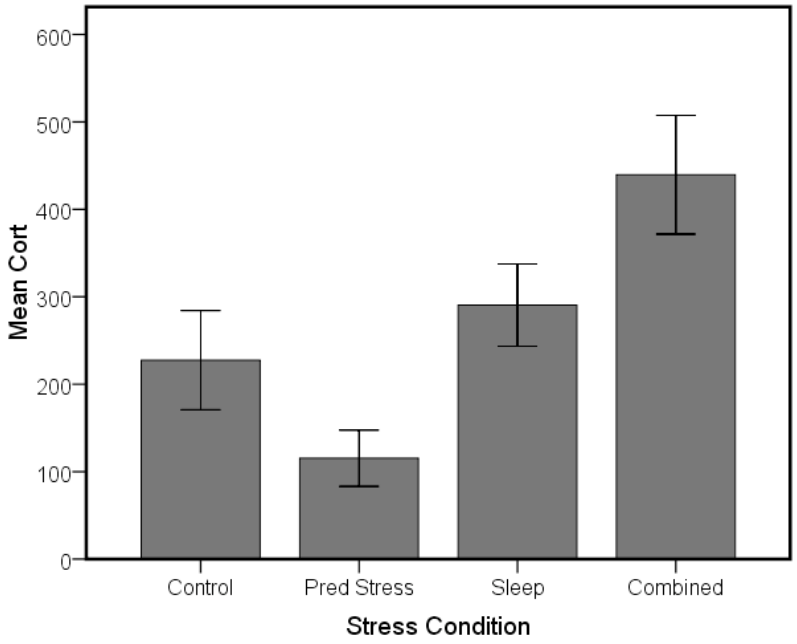
Serum Corticosterone

Blood was collected and serum corticosterone (CORT) was measured at the conclusion of the experiment after animals were sacrificed on experimental day 49.

There was a significant effect on serum corticosterone levels by condition to which the animals were exposed during adolescence ($F[3, 36] = 26.42$, $p < .05$) (see Table 4). Corticosterone levels in the combined condition were

significantly greater than all other conditions. There was no significant difference between the control and sleep disruption groups. Corticosterone levels in the predator stress condition were significantly less than all other groups (Figure 1) (see Table 5).

Figure 1. Corticosterone levels by condition



The results for serum corticosterone indicate that exposure to a combination of predator stress and sleep disruption during adolescence had a greater effect than either stressor alone. Animals in the predator stress condition displayed lower stress hormone levels than animals in all other conditions. There were no differences observed between animals in the sleep disruption and control groups.

Predicted direction for CORT: Combined > Sleep = Predator > Control

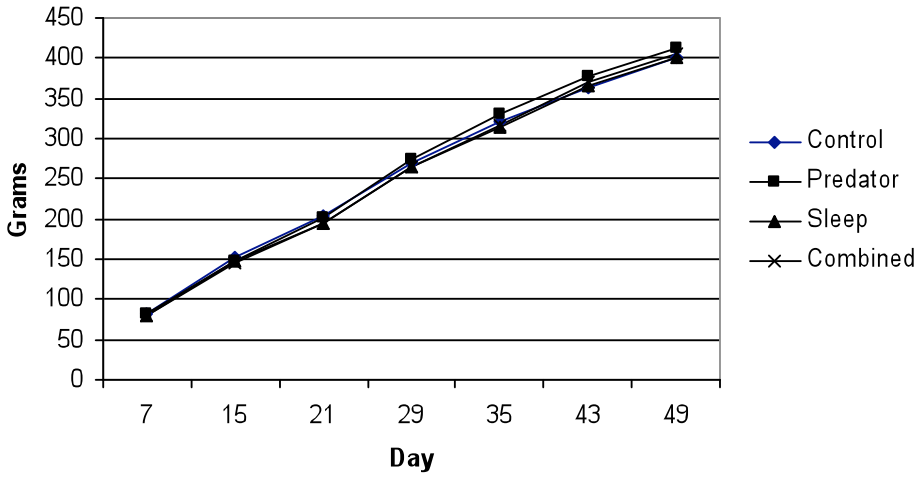
Observed direction of CORT: Combined > Sleep = Control > Predator

Hypothesis partially confirmed.

Body Weight

Body weight was measured every two days during the morning to assess the general health of animals and to track any differences based on condition. Animals gained weight from the beginning to the end of the experiment ($F[6, 216] = 2468.85$, $p < .05$). Body weight did not vary significantly based on condition at any point during the experiment (Figure 2) (see Table 7).

Figure 2. Daily body weight by condition



Rats consumed significantly more food from the beginning of the experiment to the end, as expected ($F[5, 80] = 19.72$, $p < .05$) (see Table 9). There were no significant food consumption differences based on condition throughout the experiment (see Table 10).

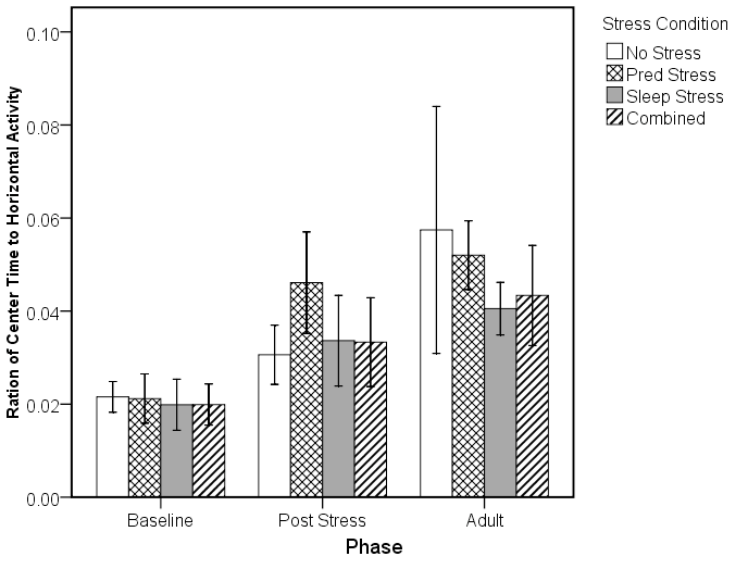
Open-Field Activity

Open-Field Activity was measured at three time-points during the experiment: at Baseline on experiment day 6 (prior to administration of stress manipulations), immediately after the stress administration procedures on day 21 (Post-stress), and at the conclusion of the two-week maturation period on day 35

(Adult). Center time, measured in seconds, was assessed as an index of anxiety-like behavior. Horizontal activity also was measured.

The predator stress group of animals displayed both increased center time and horizontal activity at the post stress and adult phases (see Tables 12, 13, 14, and 15), revealing an increase in general movement and activity. A ratio of center time to horizontal activity produced no significant effect of condition—an indication that increases in measures considered independently were artifacts of general movement increases and not markers for increased anxiety (see Table 19) (Figure 3).

Figure 3. Center time / Horizontal Activity Ratio by phase and condition



The results of open-field activity revealed no significant effects of condition on anxiety-like behavior (less center time) in male SD rats at any phase.

Predicted direction for center time: Control > Sleep = Predator > Combined at each phase after stress

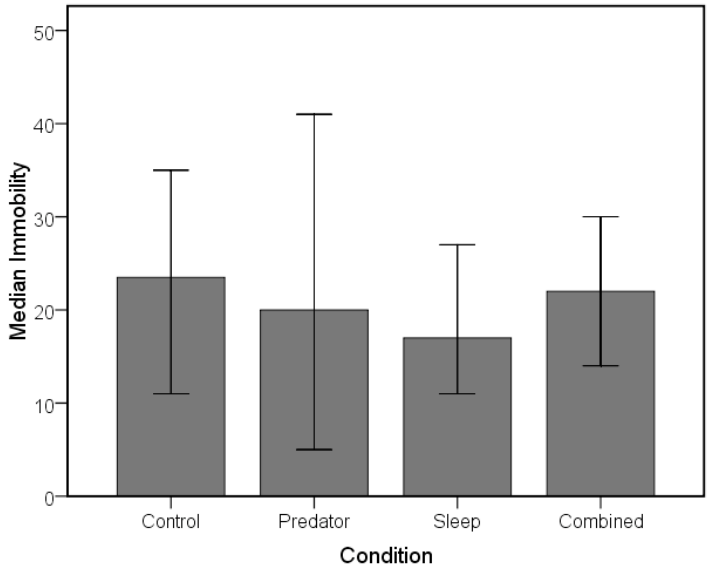
Observed direction for center time: Predator = Sleep = Combined = Control at all phases

Hypothesis not confirmed.

Forced Swim Test

The Forced Swim Test (FST) was conducted two weeks after the conclusion of the stress manipulation, when animals were in the adult phase. The purpose of the FST was to examining depression-like behavior during adulthood, based on the condition the animals were exposed to in adolescence. Analysis of median values for forced swim immobility revealed no significant effect of condition on depression-like behavior (see Table 22) (Figure 4).

Figure 4. Forced swim immobility by condition



The results of the forced swim test indicate that condition had no effect on depression-like behavior in male SD rats.

Predicted direction for immobility: Combined > Predator = Sleep > Control

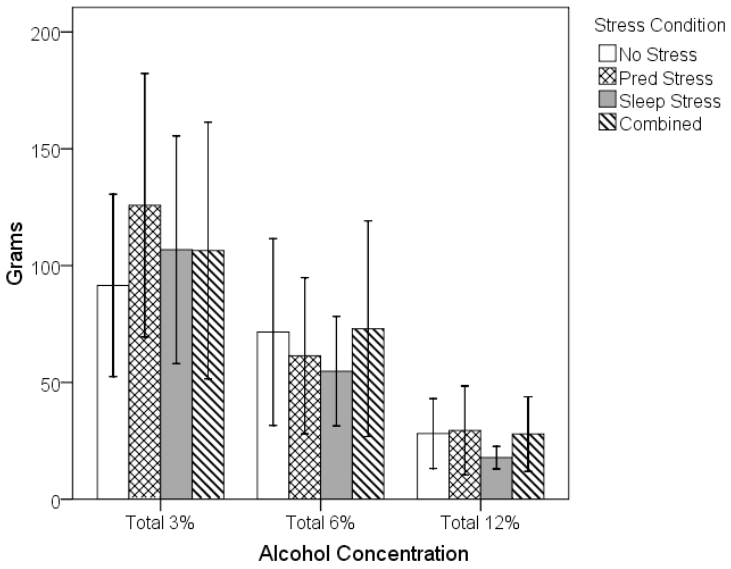
Observed direction for immobility: Control = Combined = Predator = Sleep

Hypothesis not confirmed.

Alcohol Consumption

Rats were given continuous access to ethanol at 3%, 6%, or 12% concentrations beginning during the adult phase on experiment day 38, for three days at each concentration. Alcohol intake did not differ between animals exposed to the four conditions (see Table 24). There also were no significant differences in alcohol consumption among the four treatments at each concentration (see Table 24) (Figure 5).

Figure 5. Ethanol consumption by condition



The results of alcohol consumption in this experiment revealed that adult male SD rats responded in the same manner, regardless of condition during adolescence.

Predicted direction for alcohol: Combined > Predator > Sleep > Control

Observed direction for alcohol: Control = Predator = Sleep = Control

Hypothesis not confirmed.

Discussion—Experiment 1

The purpose of Experiment 1 was to examine the effects of stressors during adolescence in male Sprague-Dawley (SD) rats on subsequent measures of mental health during adulthood. This experiment was a feasibility study designed to accomplish several goals:

1) to establish a basis by which to estimate the logistic and personnel requirements of a larger-scale study

2) to conduct procedures previously untested in the laboratory and refine techniques and processes

3) to train laboratory personnel and assistants on the specific procedures required if this work

4) to establish that the predator stress, sleep disruption, and forced swim procedures were reasonably possible to conduct in the same experiment

The study was conducted utilizing only male rats of a single strain to reduce variability based on genetic factors and isolate effects to experimental manipulation. The results indicated that male SD rats displayed no differences based on condition (control, predator stress, sleep disruption, combined stress) in body weight, center time, forced-swim immobility, or alcohol consumption. Contrary to predictions, animals in stress conditions displayed no increased alcohol consumption or depression-like behavior.

Serum corticosterone levels after sacrifice were significantly different based on conditions to which animals were assigned. As predicted, the animals in the combined condition had the highest concentrations of serum

corticosterone, suggesting that the combined effect of predator stress and sleep disruption intensified stress levels, resulting in increased corticosterone production. Corticosterone levels did not differ between control animals and sleep-disrupted animals, and unexpectedly, both were significantly greater than animals in the predator stress condition. Animals in the predator stress condition displayed the lowest levels of serum corticosterone concentration. It is noteworthy that the blood samples were taken almost a month after the stress phase of Experiment 1.

The presence of a corticosterone effect indicates that the selected stressors had an effect on the experimental subjects, but there were no significant effects of condition on body weight, center time, forced swim immobility, or alcohol consumption. Experiment 1 was a small study designed to evaluate logistics required for a larger experiment, train personnel, and practice experimental techniques. A single strain and single sex were used to minimize variance based on other factors that would be the focus of Experiment 2. Experiment 2 was designed to address questions impossible to answer with a single strain, single sex model such as Experiment 1. The second experiment was designed to consider the effect of individual differences on the expression of stress effects in varying conditions, to include sex and strain. Experiment 2 also included changes designed to improve the study: (1) assessment of FST was automated to increase sensitivity and reliability; and (2) addition of a stressor several days before sacrifice.

EXPERIMENT 2

Overview

The purpose of Experiment 2 was to examine the effects of stressors during adolescence in male and female Sprague-Dawley (SD) and Long-Evans (LE) rats on health-related behaviors in adulthood. Sex and genetic strain were of particular interest in Experiment 2. The procedures were similar to those utilized in Experiment 1 except for the automated assessment of forced swim activity and the addition of a brief, mild stressor several days before sacrifice.

Hypotheses

There were four major hypotheses based on the domains of the dependent variables: (1) serum corticosterone levels; (2) anxiety measure – center time; (3) depression measure – forced swim test performance; and (4) ethanol consumption.

Hypothesis 1: Serum Corticosterone

Because the HPA axis activated in response to stress and corticosterone is elevated in response to HPA activity (Belz et al., 2003; Hennessey, 1991; Pham et al., 1999b; Selye, 1973), it was hypothesized that serum corticosterone levels would be highest in animals previously stressed and lowest in animals in the control condition. For all animals, the expected direction of corticosterone levels from greatest to least was expected to be combined, predator stress, sleep disruption, and control. Faraday (2002) found that daily stress resulted in elevated corticosterone levels in SD rats when compared to LE rats; therefore, it

was expected that stressed SD rats would have higher concentrations of corticosterone.

Hypothesis 2: Open Field Locomotion (Center Time)

It was hypothesized that stressed animals would exhibit different locomotor behavior when compared to non-stressed animals and generally less time in the center of the open field. Faraday (2002) and Pohl et al. (2007) reported that young male rats exposed to sporadic severe stress exhibited more anxiety responses than non-stressed rats. In the same experiment, female rats exposed to sporadic severe stress and chronic mild stress displayed more behaviors modeling anxiety and depression than non-stressed rats. Based on the results of the previously mentioned experiments, male rats of both strains in the predator stress condition were expected to spend less time in the center of the open field than stressed females at each phase after the stress period. In terms of strain, SD rats were generally expected to demonstrate less open field behavior (Faraday, 2002). However, it was unclear what the effect of multiple strains and sexes would have on open field behavior and locomotion. Based on previous reports, male rats in the sleep disruption condition were expected to be less affected. Female rats in the predator stress and sleep disturbed conditions were expected to spend somewhat less time in the center of the open field than non-stressed female rats, but the relationship to center time in comparison to male rats was unclear.

Hypothesis 3: Forced Swim Immobility

It was hypothesized that there would be differential responses to stress based on stressor and sex. Based on Pohl et al. (2007) and Faraday (2002), it was expected that previously stressed female rats of both strains would display greater immobile behavior during the forced swim test than male rats, because it has been observed that female rats exhibit more depression-like behaviors. Pohl (2007) used reduced or comparatively low sucrose consumption as an indicator of depression-like behavior in rats and shows that females display more depression-like behaviors than males when stressed.

Hypothesis 4: Alcohol Consumption

It was hypothesized that animals in stressed conditions would generally consume more alcohol than non-stressed animals. SD rats were predicted to consume significantly more alcohol than LE rats. These hypotheses were based on Pohl (2007) and Faraday (2002) in which different types of stress have differential effects based on sex and strain. Based on human data which shows that men drink more than women, it was hypothesized that male rats would consume more alcohol than female rats.

Methods

Experimental Design

The design for Experiment 2 was: 2 (male, female) x 2 (SD, LE) x 4 (no stress, predator stress, sleep disruption, predator x sleep) mixed model. The total sample size in this experiment was 160 animals (10 animals / 16 cells). To obtain a more sensitive measure of the effects of the experimental conditions,

two forced swim procedures were added: one immediately after the stress administration phase. The forced swim test at the adult phase remained, and an additional forced swim test was added after the administration of a novel stressor near the end of the experiment. A novel stressor (restraint) was administered on experimental day 47; 27 days after the last day of stress administration to observe responses to a completely novel stressor based on stress history.

Subjects

The subjects were 160 animals, from Charles River Laboratories, approximately 25 days old at the beginning of the experiment. Animals at the beginning of Experiment 2 were 3 days older than animals at the beginning of Experiment 1 to ensure that they were adults at the time when the post-stress dependent measures were recorded (Spear & Brake, 1983; Ojeda & Urbanski, 1994; Faraday, Elliott, & Grunberg, 2001). SD rats were selected because they are a general-purpose experimental model most commonly used in stress experiments and they are not bred for any particular genetic characteristics. LE rats were selected because they have shown different responses to stress and drug administration than have SD rats (Faraday, 2002). The LE rat also has different phenotypic characteristics (color coat, skin, and eye pigmentation) that reflect underlying genetic differences from the SD rat. These strain differences are not analogous to human ethnic differences, but they provide a model that includes genetic differences in physical coloration. At the beginning of the experiment, the animals weighed an average of 78 grams.

For Experiment 2, animals were matched based on sex and strain, and then were randomly assigned to control, predator stress, sleep-disturbance, or combined predator stress/sleep disruption conditions. All other parameters (i.e., housing, food, water, light cycle) were identical to Experiment 1.

Independent Variables

The independent variables were the same as in Experiment 1.

Dependent Variables

The dependent variables were identical to the dependent variables in Experiment 1.

Procedure

Experiment 2 was conducted in five main phases: baseline, stress, post-stress, adult, and novel stress (see Table 3). Only body weight and food consumption were measured during the stress period. Other measures of corticosterone, anxiety-like behavior, depression-like behavior, and alcohol consumption were measured after the cessation of stress. The data analysis for most dependent variables was conducting accounting for four phases: baseline, post-stress, adult, and novel stress. Behavioral measures to assess anxiety-like and depression-like behaviors were made during baseline, immediately after stress manipulation, during adulthood, and after a novel stressor. At the conclusion of the experiments, animals were euthanized by LAM personnel (carbon dioxide inhalation euthanasia from a compressed gas cylinder), decapitated, and disposed of in accordance with university animal use policies.

Baseline

The baseline phase lasted for six days. The rats were 25 days old upon arrival and were acclimated to the facility for three days. During the baseline phase, the rats were randomly assigned to either no stress, sleep disruption only, predator stress only, or sleep plus predator stress conditions (combined). The animals were then matched by sex and strain, pair-housed, and placed in two separate rooms. Room 1 housed animals in the control condition and the predator stress-only condition. Room 2 housed animals in the sleep disruption condition and the combined (sleep and predator) condition. Each rat was handled for 3 - 5 minutes for the first three days after arrival to familiarize them with human contact and minimize stress of handling during later stages of the experiment. The animals were divided into two even cohorts for logistical purposes, and the manipulations were staggered by two days between cohorts. (All following references to the timing of experimental manipulations refer to Cohort 1 followed identically after two days by Cohort 2.) Animals were placed in open-field chambers on the fourth day after arrival for acclimation to the locomotor apparatus, and a baseline measure of open-field activity was obtained on day five. Body weight, food consumption, and water consumption were measured weekly for the duration of the experiment. Ambient sound levels of each housing room were measured on the day the animals arrived to ensure there was not interference with the sounds manipulated during the experiment. Temperature and humidity also were measured on the days that body weights

were recorded to ensure a consistent and healthy living environment for the animals.

Stress Phase (Adolescent)

The stress phase began on day seven, with rats 31 days old, and was 14 days in duration. The animals assigned to the predator stress condition were removed from their housing rooms and were transported to a separate procedure room for the stress procedure. The predator stress-exposed rats were placed in the presence of fox urine and unpredictable stimuli (e.g., noise, light, cage shaking) for 10 minutes each day. Individual cotton balls were each soaked with 15 milliliters of fox urine and placed inside empty Plexiglas cages (42.5 x 20.5x 20 cm) with plastic, filtered tops. The white overhead light was kept illuminated throughout the entire procedure. For each day of the 14-day stress manipulation, the cotton balls were placed in different sections of the cages to reduce the likelihood of habituation or place preference. Additional novel stressors, such as bells, clapping, flashing lights, and whistles were used in conjunction with the fox urine to create an unpredictable, more stressful environment. Personnel administering predator stress wore dark-colored scrubs, and not their normal white lab coats, when administering predator stress. The wear of different clothing served two purposes: to reduce the possibility of fox urine contaminated clothing affecting animals not assigned to the predator stress condition upon return to the housing rooms and to take advantage of any conditioned responses to clothing of the individuals administering the stress.

Rats in the sleep disruption-only condition were exposed to recorded noises obtained from open-source Internet sites (e.g., banging, talking, coins, doors slamming) during their low-activity period on a nightly variable interval schedule. There were eight recorded noises lasting between six seconds and one minute-ten seconds. The selected noises were relatively common environmental sounds of varying frequencies (Rabat et al., 2006, 2005, 2004). The ambient noise level in the sleep disruption room, before sound manipulation, was 59 decibels (dB). The sound level of the recorded noises ranged from 65 dB to 80 dB. Total exposure to noise during a given hour was 5 minutes, 46 seconds. Sounds began 30 minutes after the animals' dark period began and played hourly for 10 hours. Total exposure to sound on each night totaled 54 minutes, 6 seconds. Animals in the sleep disturbed condition also were disturbed by laboratory personnel several times daily to interrupt active period napping, by measuring and recording data, talking in the room, changing cages, measuring water and food, moving cages, and rotating cage racks. Sound levels were measured and verified using a Larson-Davis Sound Level Meter, Model 2800A, with a microphone placed in the vicinity of the animal cages for measurement.

Post-Stress Phase

The post-stress phase began on day 21 of the experiment with the rats at 45 days old. Post-stress open field locomotion was performed on day 21. The initial trial of the forced swim procedure was conducted on day 22, and the first forced swim test was conducted on day 23 with animals that were 47 days old.

Six Plexiglas cylinders (30.48 cm diameter x 60.96 cm height) were filled to 30 cm with a mixture of hot and cold water until the temperature was approximately 26-27 degrees Celsius in each cylinder. The room temperature in the swim test laboratory was maintained at between 21-24 degrees Celsius. Although additional forced swim tests were conducted, the 15 minute initial trial was performed once and was not repeated. The animals were placed into the water-filled containers and swam for 5 minutes without assistance. The rats' behavior was measured by a computer program linked to ceiling-mounted video cameras and a video tracking system (Anymaze Video Tracking Software, Stoelting Co.) and were evaluated based on the time (in seconds) they spent immobile, indicated by lack of swimming or movement. Use of the Anymaze video tracking software was a major improvement on the method of observation utilized in Experiment 1. The first experiment required at least three individuals to observe animals and track behaviors, as well as two individuals to exchange animals between trials. The process was personnel intensive, and use of several individual raters introduced unwanted variance. The use of computer tracking software in Experiment 2 reduced the personnel requirement to two and ensured that variance was minimized, as the computer tracked and recorded data for six animals at once.

The animals were then removed from the containers, dried with towels, and placed under a heat lamp for approximately 10 minutes. The remainder of the forced swim procedure in Experiment 2 was identical to that of Experiment 1. At the end of the post-stress phase, the rats were 60 days old.

Adult Phase

After the 2-week post-stress period, the adult phase began on experiment day 35 with measurement of open field activity on the first day of the phase and weekly body weight and food consumption measurements until the end of the experiment. At 60 days of age, animals were at least 5 days beyond the point considered adulthood in rats. During the adult phase, forced swim immobility and voluntary consumption of alcohol were measured. The forced swim procedure was conducted on the second day of the adult phase and was identical to the 5 minute procedure conducted during the post-stress phase.

Beginning on experiment day 38, the rats were given continuous access to ethanol in their home cages at three concentrations: experimental days 38 – 40: 3%; experimental days 41 – 43: 6%; experimental days 44 – 45: 12%. Voluntary alcohol consumption was measured on each day of alcohol administration. Access to water was unrestricted for the entire experiment, including the period of alcohol consumption. Water consumption was measured during the same period when alcohol was administered and measured.

Novel Stress Phase

On experiment day 47, one day after cessation of 12% ethanol administration, the animals were administered restraint stress for 20 minutes, using Broome Rodent Restrainers (Harvard Apparatus, Items 520486/520494), and were then placed in an open-field chamber for 1 hour to obtain a measure of behavior after an acute, novel stressor. On experiment day 48, the animals were restrained and then placed in water-filled Plexiglas containers for a 5 minute

forced swim test after a novel stressor. On experimental days 49, 50, and 51, the animals were again administered Ethanol in concentrations of 3%, 6%, and 12%, respectively, each day to measure Ethanol consumption in response to a novel stressor. One day after cessation of ethanol administration, the animals were sacrificed on experimental day 54, and blood was collected and centrifuged to determine serum corticosterone levels.

Body weight was measured using Sartorious electronic scales (Sartorious AG, Goettingen, Germany) programmed to measure and average multiple weight readings within several seconds to account for errors caused by movement of the animal in the apparatus. Food and water consumption were calculated by weighing food weekly and water when alcohol was administered.

Data Analytic Strategy for Experiment 2

The data analytic strategy for Experiment 2 included repeated-measure ANOVAs to analyze sex and strain across each phase of the experiment. Post hoc analyses were conducted when there were main effects observed. All other analyses were identical to those detailed in Experiment 1.

RESULTS

P values in the document text are presented as (< 0.05) for each significant finding, regardless of actual p value. Statistical tables and analyses with complete details of each analysis are presented in Appendix A. Figures and graphs are listed in the document text.

Serum Corticosterone Level

Trunk blood was collected on day 52 of the experiment, after completion of all measurements and manipulations. Immediately after decapitation, blood was collected in plastic tubes, centrifuged, and serum was stored in a low temperature freezer until it was assayed for corticosterone.

Overall, there was a significant effect of stress on serum corticosterone levels ($F[3, 143] = 4.77$, $p < .05$) (see Table 25). Corticosterone levels in the predator stress, sleep disruption, and combined conditions were each significantly greater than controls, but were not significantly different otherwise (Figure 6) (see Table 26). Female rats displayed higher corticosterone levels than male rats overall ($F[1, 143] = 21.19$, $p < .05$) (see Table 27). There were also sex based differences at each condition, with males and females displaying varying levels of corticosterone across conditions ($F[3, 143] = 2.95$, $p < 05$) (Figure 7). Female rats displayed corticosterone levels in the combined condition that were significantly higher than corticosterone levels in the control condition (Figure 8) (see Table 28). Male rats displayed different characteristics, with corticosterone at the highest levels in the sleep disruption condition and the predator stress condition (see Table 29).

Figure 6. Corticosterone Level by Condition

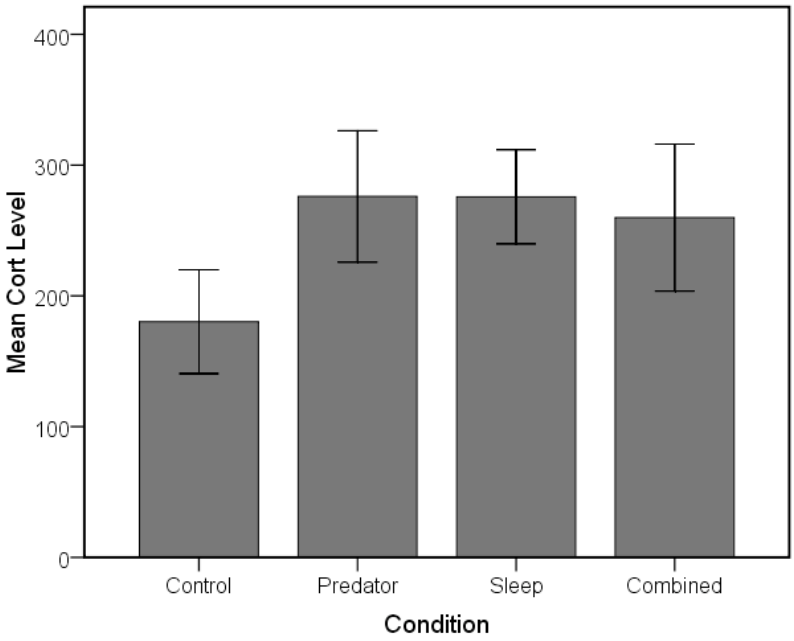


Figure 7. Corticosterone Level by Sex x Condition

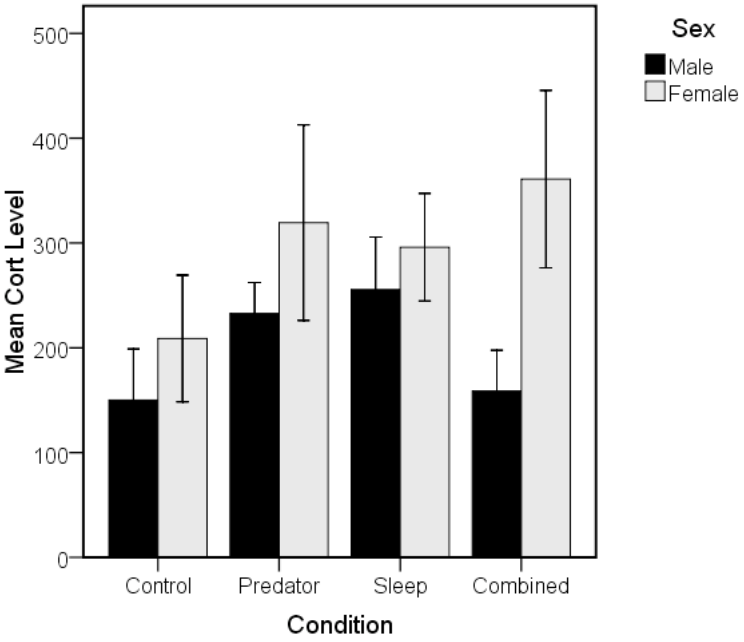


Figure 8. Corticosterone Level by Sex—Females

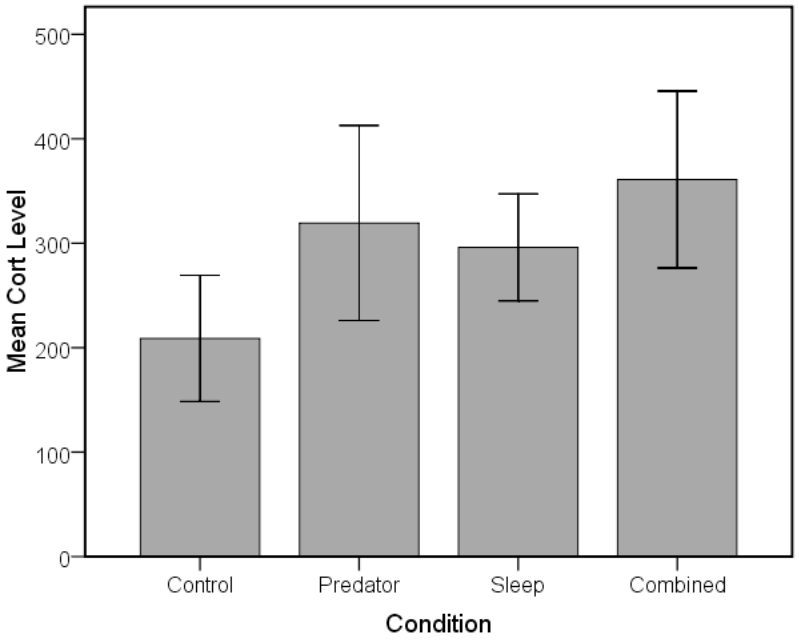
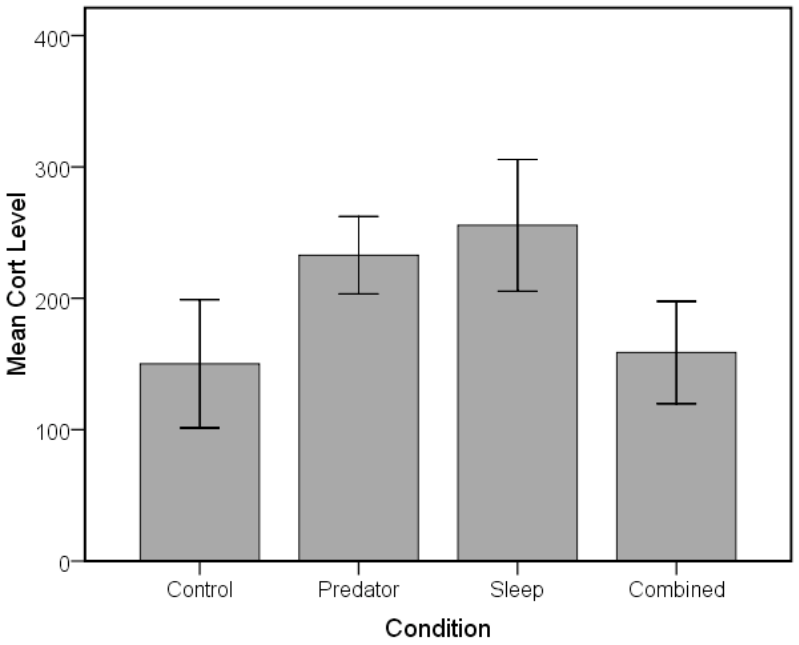


Figure 9. Corticosterone Level by Sex—Males



The current results of serum corticosterone reveal that stress conditions and sex have an effect on the production of stress hormones in rats. Sleep disruption and predator stress had the greatest effect in male rats, while the combined stress condition had the greatest effect on stress hormones in female rats.

Summary of results for corticosterone:

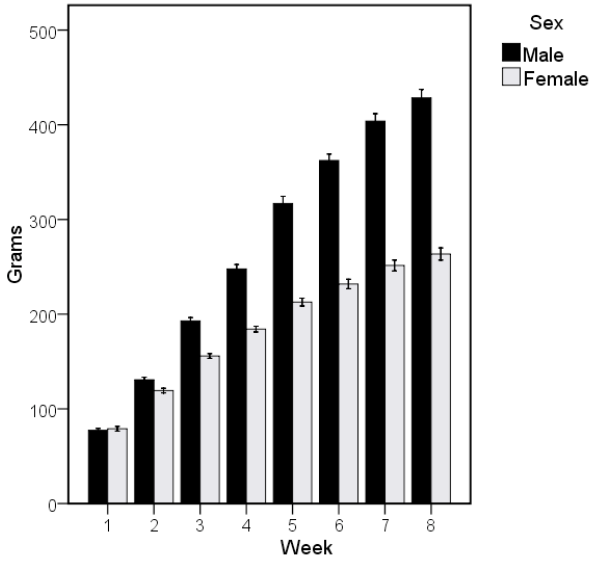
- Stress exposure resulted in higher corticosterone levels
- Overall, all stress conditions resulted in greater biochemical stress reactions than the control condition
- Females display generally higher levels of CORT than males, particularly when exposed to multiple stressors

Body Weight

Body weight was measured once weekly for the duration of the experiment. All rats gained weight over time ($F[7, 1008] = 8119.15$, $p < .05$ [see Table 36]), and males weighed more than females ($F[1, 44] = 825.29$, $p < .05$ [see Table 37]). Male rats gained weight more rapidly than female rats over time ($F[7, 1008] = 891.55$, $p < .05$) (see Table 36), and body weight differed based on the strain of the animals and time measurements were taken ($F[7, 1008] = 34.11$, $p < .05$). Body weight also differed within each strain by sex over time ($F[7, 1008] = 7.48$, $p < .05$) (see Table 36). The results for body weight confirm that animals gained weight over time, although they gained weight at different rates based on sex, strain, and the week they were measured. Overall, body weights were similar during the first week of the experiment, after which male rats

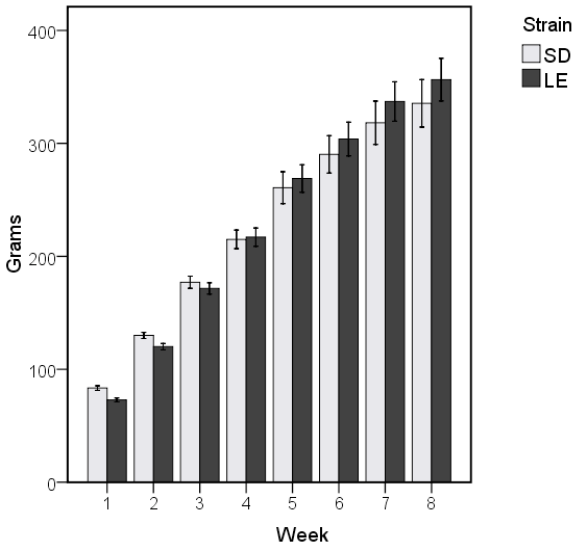
weighed significantly more than female rats on each subsequent week ($F[7, 1008] = 891.55$, $p < .05$). The body weight difference between male rats and female rats increased progressively from 11.40 grams at week 2, to 164.98 grams during the last week of the experiment (Figure 10) (see Table 33).

Figure 10. Weekly body weight in grams by sex



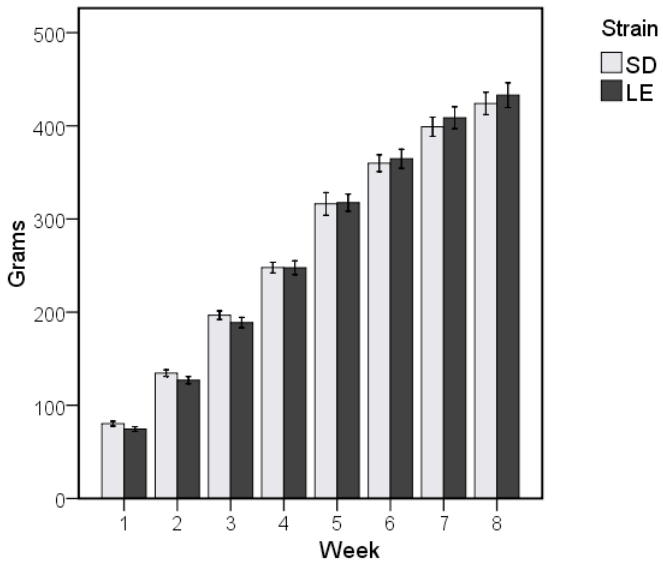
In terms of strain, SD rats weighed significantly more than LE rats in the first three weeks with no difference during weeks 4 and 5 (see Table 35). In the final three weeks of the experiment, LE rats weighed significantly more than SD rats.

Figure 11. Weekly body weight in grams by strain



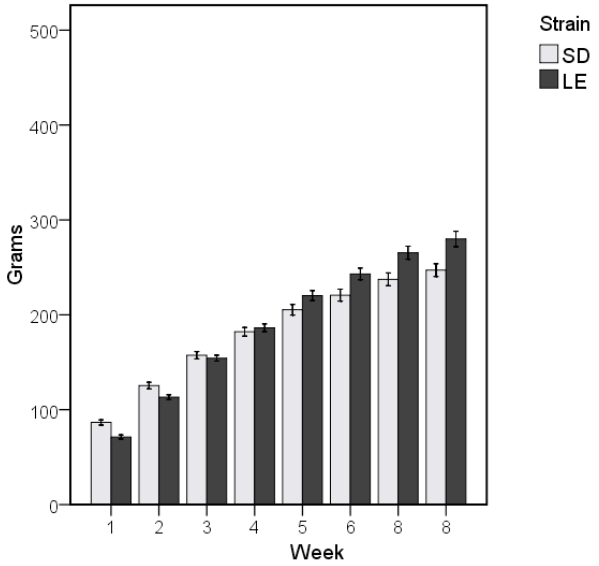
There were differences over time based on sex and strain, with male SD rats weighing more that male LE rats during the first three weeks (baseline and stress period) with no difference during the last 5 weeks (see Table 39) (Figure 12).

Figure 12. Body weight by sex and strain (male)



In contrast, female SD rats weighed more than female LE rats for only the first two weeks (baseline and first week of stress) (see Table 40). There was no difference between female SD and female LE rats during the 3rd and 4th weeks of the experiment, but LE female rats weighed significantly more than SD female rats during the last two weeks of the experiment (Figure 13).

Figure 13. Body weight by sex and strain (female)



The time x strain x sex interaction ($F[7, 1008] = 7.48$, $p < .05$) illustrates differences in the trajectory of body weight based on sex and strain, with females displaying the most notable differences between strains from during the initial and final weeks of the experiment (Figures 12 & 13) (see Table 34).

Overall, male rats weighed more than female rats regardless of treatment condition or strain ($F[1, 44]=825.29$, $p <.05$) (see Table 37). Collapsing across sex, SD rats weighed significantly more than LE rats in the initial weeks of the experiment, with LE rats weighing significantly more in the final weeks of the experiment (see Table 35). Male animals did not follow the overall strain trend,

because male LE and male SD rats displayed no significant differences in the final weeks of the experiment (see Table 39) (Figure 12).

Body Weight Gain

Overall, body weight gain was greatest during the initial weeks of the experiment and declined over time in all animals ($F[6, 864] = 471.53$, $p < .05$) (see Table 46). Body weight gain fluctuated between 40 grams and 50 grams for the first 5 weeks and then declined steadily for the next 3 weeks to approximately 20 grams of body weight gain in the last week of the experiment (Figure 14). Percentage of body weight gain differed depending on condition during week 5 and week 8 (Week 5: $F[3, 144] = 5.04$, $p < .05$); (Week 8: $F[3, 144] = 6.87$, $p < .05$) (see Table 47). Rats in the predator stress condition gained weight more rapidly from week 4 to week 5 than rats in other conditions and returned to a pattern more similar to the other conditions in week 6 and week 7 (Figure 15) (see Table 48). The relative increase in weight gain from week 4 to week 5 was despite the fact that animals in the predator stress condition consumed less food than control animals during the same period ($F[3, 60] = 3.12$, $p < .05$) (Figure 16). The period between weeks 4 and 5 corresponds with the second week of the post-stress phase (see Table 3). Rats in the combined stress condition gained significantly less body weight from week 7 to week 8 than did animals in each other condition ($F[3,144] = 6.87$, $p < .05$) (Figure 15) (see Table 48), while there was no significant difference in food consumption between conditions during week 8 (Figure 16) (see Table 49). Week 8 involved a novel stressor (restraint stress), alcohol consumption, open field activity observations,

and a forced swim test. Novel stress in animals previously exposed to an acute stressor resulted in a slower rate of weight gain, although food consumption remained unchanged.

Males gained more weight than females at each point during the experiment ($F[6, 864] = 74.24$, $p < .05$). Within each sex, there were strain differences in weight gain over time ($F[6, 864] = 3.29$, $p < .05$) (see Table 46). Male SD rats weighed more than male LE rats in the beginning weeks of the experiment, with no difference after week 3 (see Tables 39 and 34). Female SD rats weighed more than female LE rats during the first 2 weeks, but female LE rats weighed more in the final 2 weeks (see Table 40 and 34).

Figure 14. Weekly body weight gain

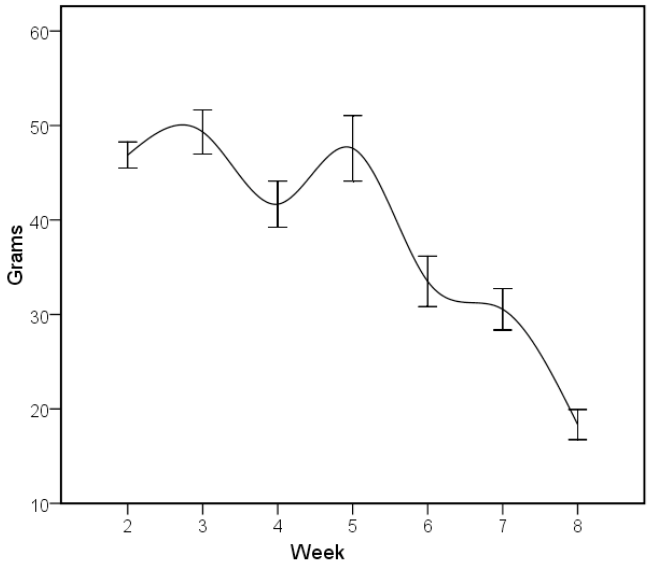


Figure 15. Weekly body weight gain by stress condition

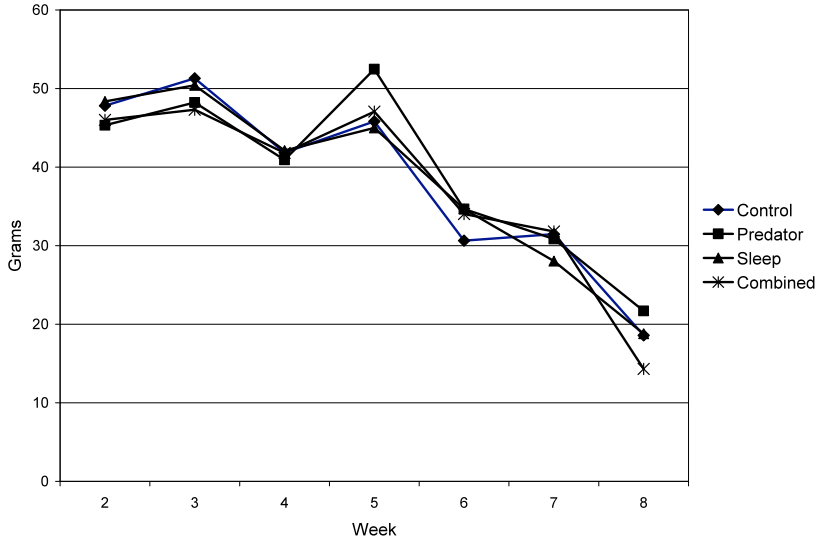
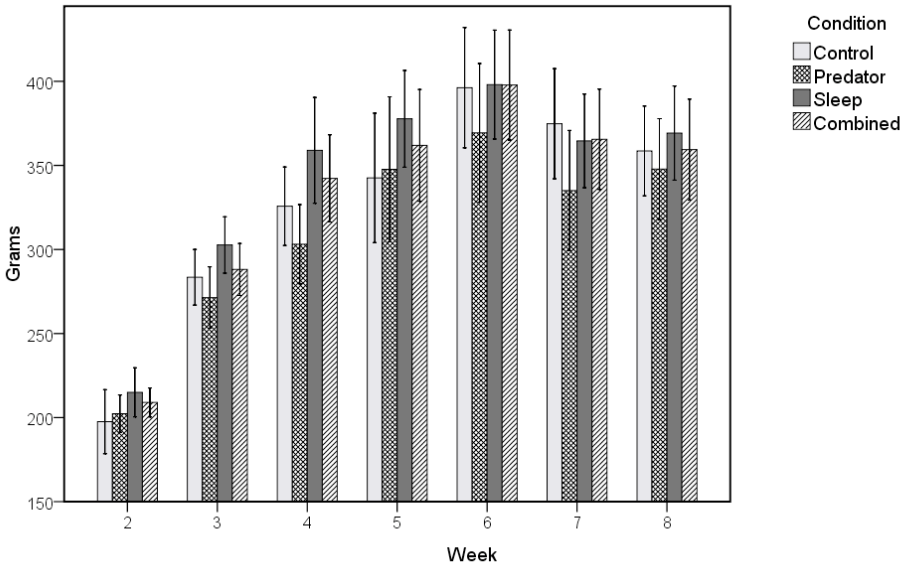


Figure 16. Food consumption by stress condition



Summary of results for body weight:

- Rats gained weight over time
- Male rats weighed more than female rats
- Rats in the predator stress condition gained the most weight during the 2nd week of stress, while consuming the least amount of food
- Rats in the combined condition gained the least weight after a novel stressor in the final week of the experiment
- Male SD rats weighed more than male LE rats in the beginning weeks of the experiment, with no difference after week 3
- Female SD rats weighed more than female LE rats during the first 2 weeks, but female LE rats weighed more in the final 2 weeks

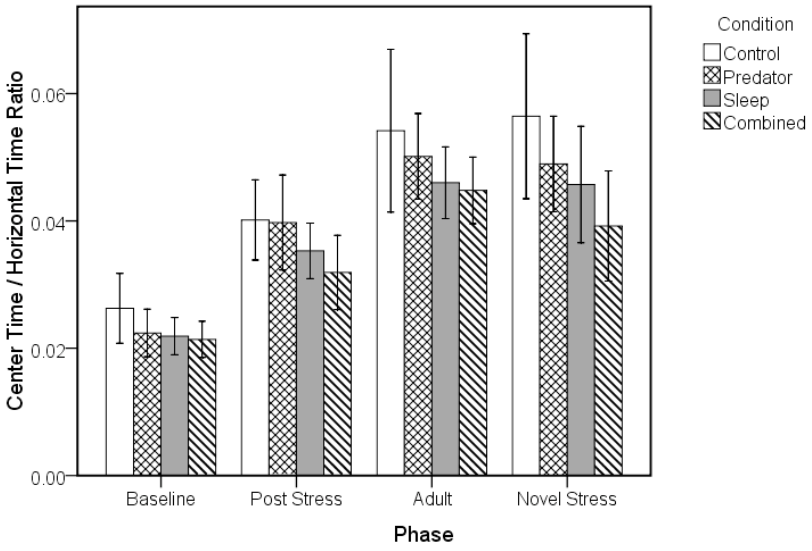
Open-Field Activity

Open-field activity was measured at four time-points during the experiment: at baseline on experiment day 5 (prior to administration of stress manipulations), immediately after the stress administration procedures (Post-stress), at the conclusion of the two-week post-stress rest period on (Adult phase), and immediately after the administration of a novel stressor (Novel Stress). There were two open-field activity variables analyzed: center time and horizontal activity. Center time was measured as an indicator of anxiety-like behavior, with less time in the center of the open field interpreted as greater anxiety. Horizontal activity was a simple measure of health and general level of movement. Horizontal activity also was measured as a way to assess center time in relation to total movement and activity.

Center Time (Ratios)

The proportion of center time to horizontal activity did not differ significantly by condition at any phase; however, the effect of stress to decrease center time approached significance after administration of the novel stressor ($F[3, 144] = 2.65$, $p = 0.51$) (Figure 17) (see Tables 50 and 51).

Figure 17. Center time ratio by condition and phase



Center time also differed significantly over time based on sex with female rats displaying less center time than males at baseline ($F[1, 144] = 4.22$, $p < .05$), adult ($F[1, 144] = 25.46$, $p < .05$), and novel stress phases ($F[1, 144] = 33.52$, $p < .05$) (Figure 18) (see Table 50). SD rats spent less time in the center of the open field than LE rats during the novel stress phase ($F[1, 144] = 6.53$, $p < .05$) (Figure 16).

Figure 18. Center time ratio by sex

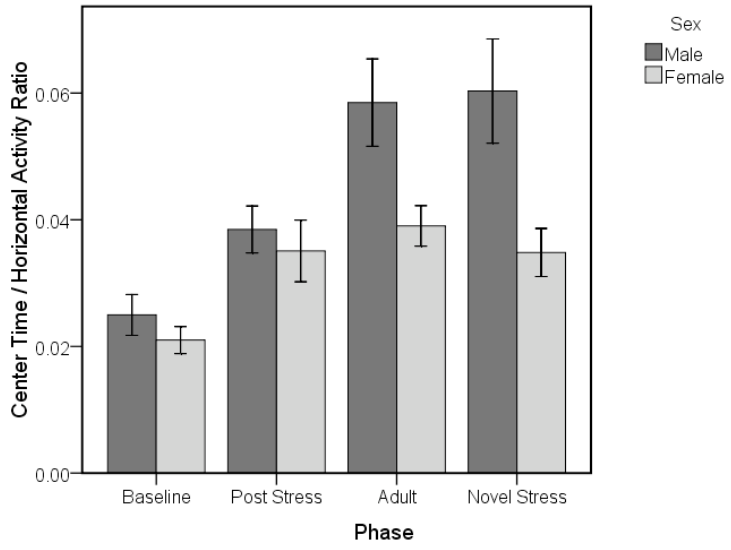
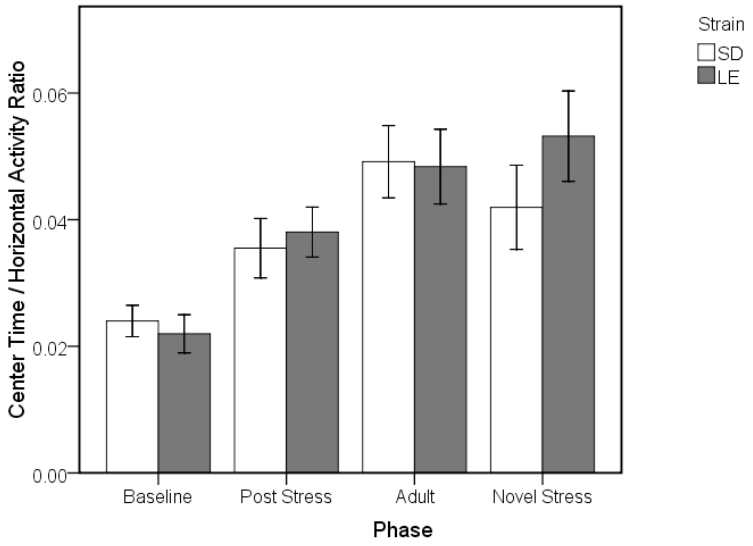


Figure 19. Open-field center time by strain



Summary of results for center time:

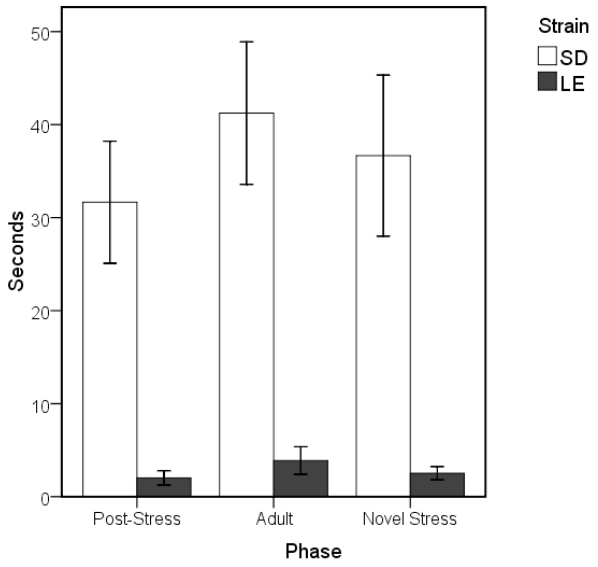
- Rats in the combined stress condition spent less time in the center (suggesting more anxiety) compared to controls
- Female rats spent significantly less time in the center of the open field in the Adult and Novel Stress phases, indicating more anxious behavior in females immediately after stress and during a different stressor later in adulthood
- SD rats spent less time in the center of the open field than LE rats during the Novel Stress phase, indicating more anxiety in SD rats after initiation of a new stressor later in adulthood

Forced Swim Test

The Forced Swim Test (FST) was conducted at three time points during the experiment to access depression-like behaviors, as indicated by relatively more immobility in the water. The initial FST was conducted one day after the cessation of predator stress and sleep disruption (Post-Stress phase). The second FST was conducted during the initial day of the Adult phase, two weeks after cessation of stress manipulations The last FST was conducted after the administration of novel stress (see Table 3).

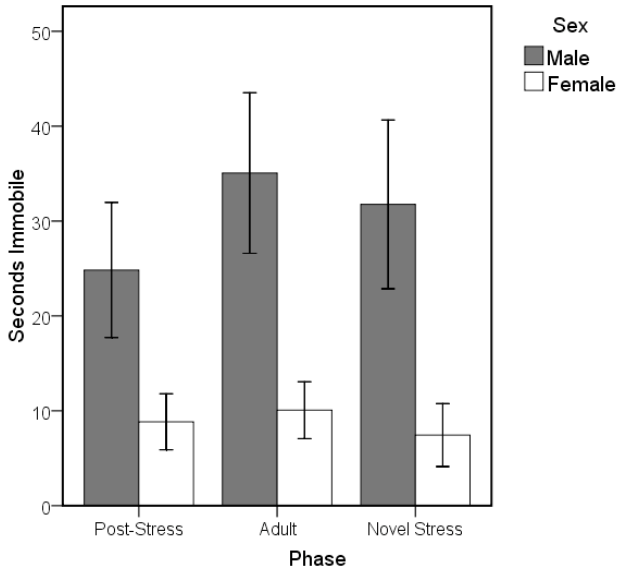
SD rats remained immobile for considerably more time than LE rats during administration of the FST during the post stress phase ($F[1, 144] = 114.26$, $p < .05$), during adulthood ($F[1, 144] = 171.30$, $p < .05$), and after the administration of a novel stressor during adulthood ($F[1, 144] = 114.80$, $p < .05$), regardless of sex or condition (See Table 58) (Figure 20).

Figure 20. Forced swim immobility by strain



Male rats were immobile for significantly more time than females during the post stress ($F[1, 144] = 33.25$, $p < .05$), adult ($F[1, 144] = 76.75$, $p < .05$), and novel stress phases ($F[1, 144] = 58.26$, $p < .05$) (See Table 58) (Figure 21).

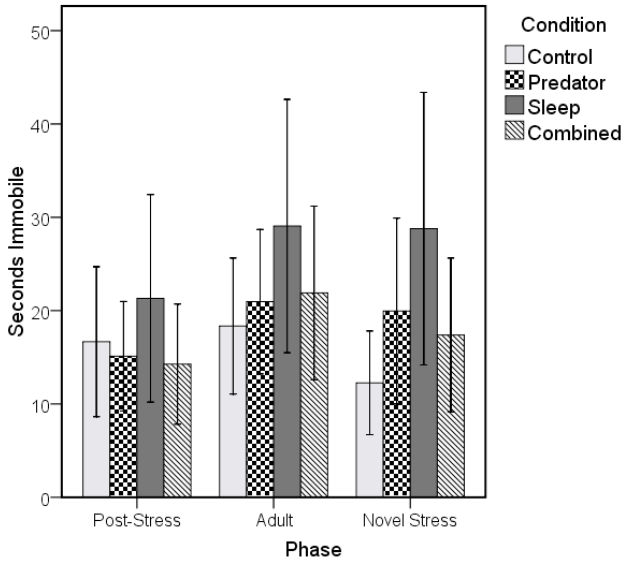
Figure 21. Forced swim immobility by sex



There was an effect of condition such that animals in the sleep condition remained immobile for significantly more time than animals in the control

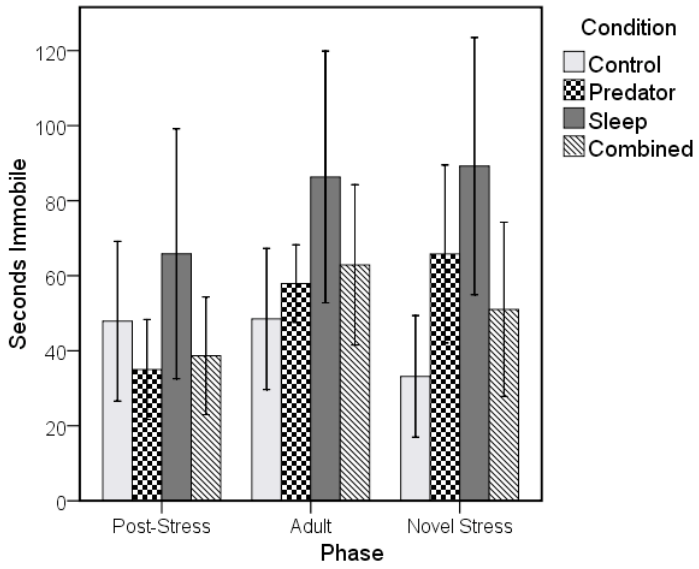
condition during the novel stress phase (F[3, 144] = 4.70, p < .05) (Figure 22) (see Tables 58 and 59).

Figure 22. Forced swim immobility by stress condition



The effects for stress condition were primarily accounted for by SD animal responses. Both SD and LE male rats were less mobile than the SD and LE female rats (Figure 21); however, SD male rats were more reactive to stress conditions, with SD males in the sleep disruption condition displaying significantly more immobile behavior than animals in the control condition during the novel stress phase (F[3,36] = 4.54, p < .05) (see Tables 61 and 62) (Figure 23). There were no significant differences by stress condition in male LE rats, female SD rats, or female LE rats (see table 61).

Figure 23. Male SD forced swim immobility by stress condition



The results for the FST indicate that there are marked differences in stress reactivity based on genetic strain. There are also sex differences, with males behaving in a manner that suggests greater stress sensitivity in general (Figure 21) and sensitivity to sleep disruption in particular in male SD rats (Figure 23). These results suggest sex-based differences in the effects of stress with sleep having a greater impact.

Summary of results for forced swim immobility:

- Animals in the sleep condition were immobile for significantly more time than animals in other conditions, indicating increased depression-like behavior as a result of sleep disruption
- SD rats remained immobile for considerable more time that did LE rats at all phases regardless of sex or condition indicating an effect of strain on increased depression-like behavior

- Male rats remained immobile for more time than female rats during the Post Stress and Novel Stress phases, indicating more depression-like behavior in males immediately after a stressor and to a different stressor later in adulthood
- SD male rats in the sleep disruption condition displayed significantly more relative immobility behavior than animals in the control condition at the novel stress phase

Alcohol Consumption

Animals were given 24-hour access to 3%, 6%, or 12% ethanol solutions, during the adult phase and after the novel stressor administration. Ethanol consumption was a face-valid measure of the extent to which stress affected consumption of alcohol. Overall, animals in the sleep disruption condition consumed more alcohol than animals in other conditions and significantly more than animals in the predator stress condition ($F[3, 58] = 3.97$, $p < .05$) (see Tables 63 and 65). The difference of alcohol consumption between sleep disruption and conditions other than predator stress was non-significant. In each condition, the animals consumed the 3% concentration in the greatest amounts ($M = 71.42$ g), followed by 6% concentration ($M = 36.61$ g), and 12% in the least amounts ($M = 15.79$ g) ($F[2, 116] = 13.99$, $p < .05$) (see Table 64) (Figure 24). SD rats consumed significantly more ethanol than LE rats at all concentrations ($F[1, 58] = 23.06$, $p < .05$).

There was also a significant sex x condition interaction ($F[3, 58] = 3.22$, $p < .05$), a significant strain x condition interaction ($F[3, 58] = 3.66$, $p < .05$), a

significant sex x strain x condition interaction (F[3, 58] = 4.95, p < .05), a concentration x strain interaction (F[2, 116] = 4.18, p < .05), and a concentration x strain x condition interaction (F[6, 116] = 2.31, p < .05) (see Tables 63 and 64).

Figure 24. Total ethanol consumption by concentration

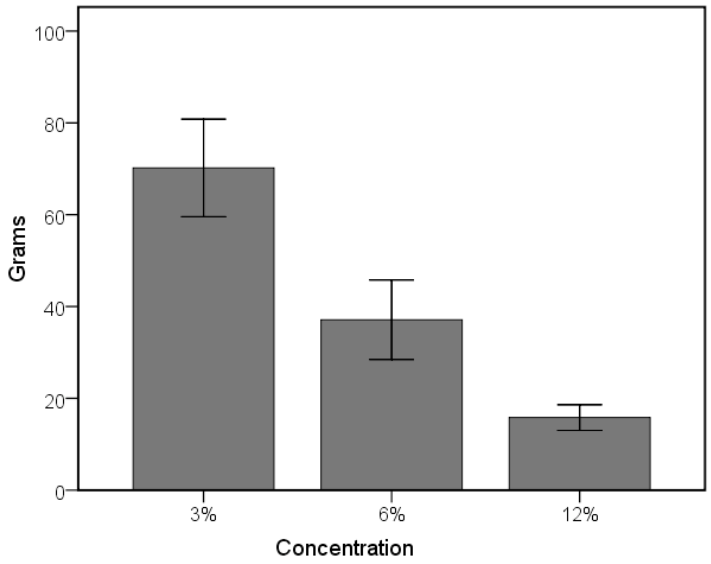
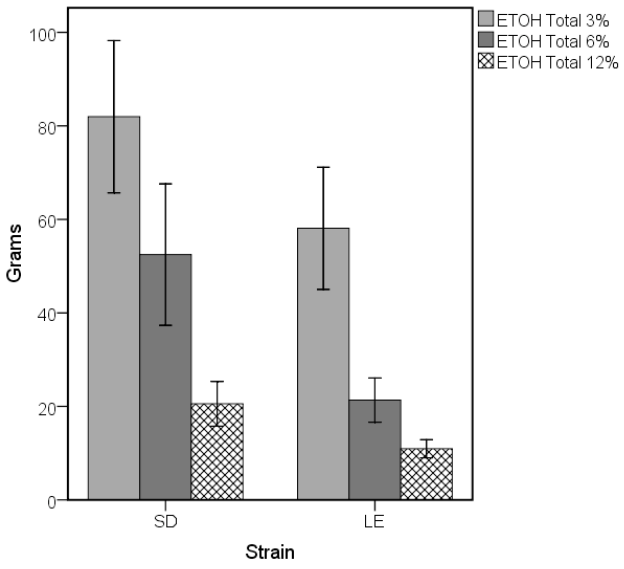


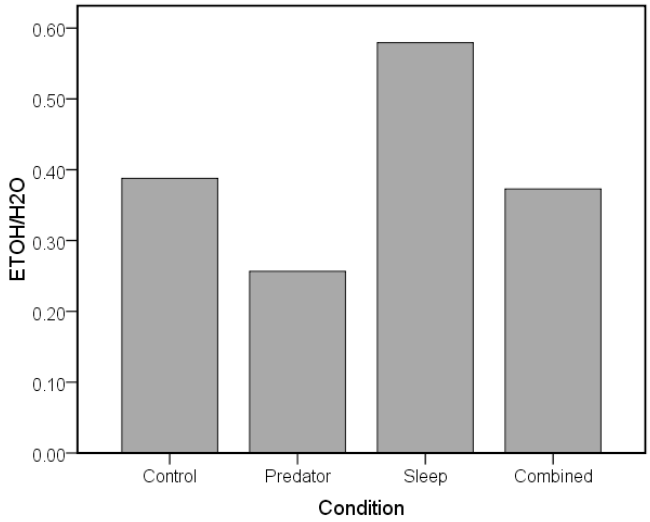
Figure 25. Ethanol consumption by strain



The amount of ethanol consumed differed significantly based on the sex of the animal and the stress condition to which assigned (F[3, 58] = 3.22, p < .05) (see

Table 63). Male rats in the sleep disruption condition consumed significantly more ethanol than male rats in all other conditions ($F[3,29] = 5.66$, $p < .05$), differing most with male rats in the predator condition (Figure 26) (see Tables 66 and 67).

Figure 26. Male Ethanol Consumption by Condition



In contrast to male rats, alcohol consumption in female rats remained relatively stable across different conditions with no significant differences (see Table 66) (Figure 27).

Figure 27. Female Ethanol Consumption by Condition

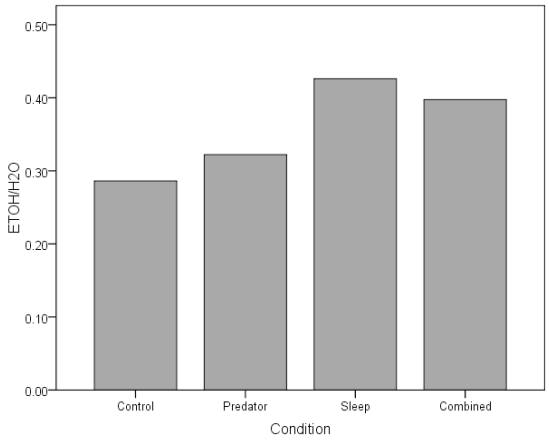
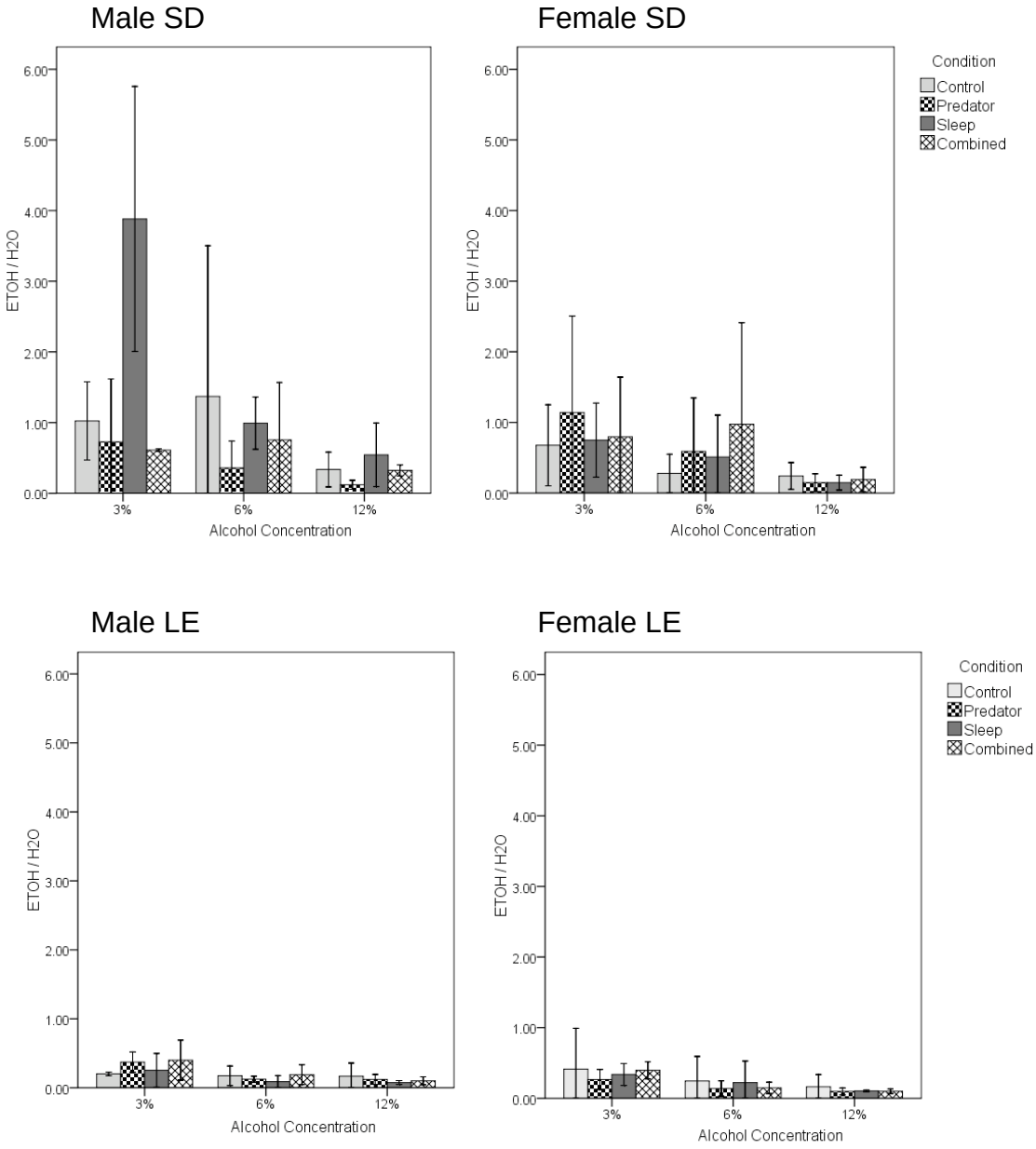


Figure 28. Ethanol Concentration by Sex, Strain, & Condition



The relative relationship of the ethanol consumed differed depending on the concentration, the strain of the animal, and the condition ($F[6, 116] = 2.31$, $p < .05$) (see Table 64). There were no significant ethanol consumption differences after the initiation of the novel stressor.

These results suggest that SD rats and male rats are generally more likely to consume more ethanol than LE rats and female rats, respectively. These results also indicate that sleep disruption is a profound factor with regard to alcohol consumption, particularly in male animals of certain strains.

Summary of results for alcohol consumption:

- SD rats consumed greater quantities of ethanol than LE rats
- Overall, sleep disruption resulted in increased ethanol consumption
- Male rats in the sleep disruption condition consumed significantly more ethanol than male rats in other conditions
- Female rats ethanol consumption did not differ between conditions

ASSESSMENT OF EXPERIMENT 2 HYPOTHESES

Hypothesis 1: Serum Corticosterone. It was hypothesized that serum corticosterone levels would be highest in animals previously stressed and lowest in control animals, with an expected direction greatest concentration to lowest being combined stress, predator stress, sleep disruption, and control.

Hypothesis 1 partially supported. The highest levels of corticosterone were in animals previously stressed compared to those in the control condition, but there were no significant differences between stress conditions. Male rats and female rats differed in corticosterone levels across conditions. Female rats displayed the highest concentration of stress hormone in the combined stress condition, while male rats displayed the highest levels of stress hormone at the sleep disruption and predator stress conditions.

Hypothesis 2: Center Time. It was hypothesized that animals in the stressed conditions would exhibit less center time than non-stressed animals. Male rats of both strains in the predator stress condition were expected to spend less time in the center of the open field that stress females at each phase after the stress period, and male rats in the sleep disruption condition were expected to be less stressed and, therefore, spend more time in the center of the open field. Stressed SD rats were expected to spend generally less time in the center than stressed LE rats.

Hypothesis 2 – Partially supported. Conservatively, there were no significant effects of condition on center time; however, combined stress animals' display of less center time at the novel stress phase approached significance. SD rats

spent less time in the center of the open field than did LE rats during the novel stress phase. Female rats spent less time in the center of the open field than did male rats at the adult and novel stress phases.

Hypothesis 3: Forced Swim Test (FST). It was hypothesized that previously stressed female rats of both strains would display greater immobile behavior during the forced swim test than previously stressed males or controls.

Hypothesis 3 – Not supported. Animals in the sleep disruption condition were immobile for significantly more time than animals in other conditions. Male rats were immobile for more time than female rats during the post stress and novel stress phases. SD rats in the sleep disruption condition displayed significantly more relative immobility behavior than controls.

Hypothesis 4: Alcohol Consumption. An interaction of sex and condition was expected, such that male rats in the predator stress condition would consume more ethanol than male rats in the sleep disruption condition. SD rats were expected to consume greater quantities of ethanol than were LE rats. It was also expected that female rats in both predator and sleep disruption would more ethanol than unstressed female rats.

Hypothesis 4 – Partially supported. SD rats consumed more ethanol overall than LE rats. **Sleep disruption resulted in an overall increase in ethanol consumption.** Male rats in the sleep disruption condition consumed significantly more ethanol than male rats in other conditions.

DISCUSSION

The purpose of this doctoral dissertation research was to examine effects of acute and chronic stress during late adolescence on subsequent indices of behavioral health during adulthood in male and female Sprague-Dawley (SD) and Long-Evans (LE) rats. The research was designed to model a militarily-relevant scenario: young soldiers under intense acute stress (as with recurring threat of injury or death) and/or chronic, non-threatening stress (as with disrupted sleep). The dependent variables of interest were serum corticosterone, body weight, open-field locomotor activity (center time), forced swim immobility, and voluntary alcohol consumption. This research included two separate experiments: Experiment 1 which was a feasibility study investigating male SD rats only; and Experiment 2 with investigated male, female, SD, and LE rats. Experiment 1 established that the logistics and chosen stress manipulations could be effectively and efficiently implemented in our laboratory and provided an opportunity to train laboratory personnel on the experimental procedures. Experiment 1 also established the selected stressors as sufficient to elicit physiological stress responses. The comparison of sex and genetic strain were of particular interest in Experiment 2. Experiment 2 provided a direct comparison of male and female rats of both SD and LE strains in order to determine the extent to which individual differences played a role in the effect of acute and chronic stress during adolescence on later expression of physiological changes, anxiety-like behavior, depression-like behavior, and alcohol consumption. In addition, Experiment 2 included changes based on the findings of Experiment 1

(i.e., automated measurement of forced swim and stress in close proximity to sacrifice).

The first major finding is the extent to which early life stress in general, has an effect on physiological markers of stress later in adulthood. In Experiment 2, each stress condition resulted in elevated corticosterone levels. That there was no difference between stress conditions was an interesting finding in that sleep disruption resulted in corticosterone levels at least as high as the predator stress and combined stress conditions. **This suggests that sleep disruption is a major stressor, even before behavioral indicators of stress are considered.** That this particular sleep disruption paradigm is an effective stressor is a major finding, because this model of sleep disruption was developed and implemented for the first time in this series of experiments. The fact that approximately 10 minutes of relatively low-level sleep disruption during each hour of the low-activity period can elicit chronic stress hormone elevations is important information with regard to possible clinical implications.

Female rats displayed higher corticosterone levels than males overall, with their highest concentrations occurring in the combined stress condition. Males, on the other hand, exhibited the highest concentrations of corticosterone in the sleep disruption and predator stress conditions. These findings highlight variable sex-based, physiological differences in response to different types of stressors, suggesting that males might be more physiologically sensitive to sleep disruption and predator stress individually, while females are physiologically sensitive to the effects of a combination of the two. An additional interesting result was that the

combined stress condition did not reveal a more robust effect than the other two stress conditions. It is unclear whether or not there was an overall buffering effect of two stressors on hormonal expression in the combined stress group.

The second major finding is with regard to the effects of the various stressors chosen on open-field activity, particularly center time, an indicator of anxiety where relatively less center time is indicative of relatively more anxiety. Center time was considered in proportion to the amount of horizontal activity displayed. This method of analysis allows for the determination of increased center time due to general increases in locomotion versus independent increases in center time with general movement and locomotion remaining constant. The latter indicates an effect of center time that can be attributed to implemented stress manipulations and not increases in overall movement.

Rats in the combined stress condition displayed less center time than rats in the control condition at a significance level of $p = .051$. Cohen (1994) cautions against discarding potentially meaningful experimental findings strictly on the basis of significance level, particularly when results are within fractional margins of achieving statistical significance. Utilizing Cohen's approach of using confidence intervals, the condition effect is within a significant range at the novel stress phase (see Table 51). At the least, animals previously exposed to combined stressors show a greater tendency toward higher anxiety when exposed to new stressors later in life, as indicated by less time in the center of the open field. Another interesting and statistically significant finding regarding center time is that female rats spent less time in the center of the open field than

did males during the adult and novel stress phases, indicating that females are more likely to have anxiety long after cessation of stress and later in life when exposed to new stressors. There also was a strain difference, as SD rats spent less time in the center of the open field during the novel stress period, suggesting more anxiety in SD rats than LE when exposed to novel stress even long after maturing to adulthood. These findings make compelling arguments for individual differences in the expression of anxiety responses.

The third major finding is with regard to immobility during the forced swim test (FST), a marker for depression. Greater proportions of immobility indicated greater depression-like behavior. **The sleep condition was a major factor, with animals in the sleep condition exhibiting an overall greater degree of immobility, indicating increased levels of depression-like behavior relative to other conditions.** A somewhat unexpected finding was that SD rats displayed immobility/depression-like behavior at a rate roughly 10x that of LE rats at each point of measurement regardless of other factors. This reveals that SD rats' baseline level of depression-like behavior exceeds that of LE rats and should be considered when making any conclusions. In addition to profound strain differences, sex differences within strain also were observed. Male rats remained immobile for more time than did female rats during the post stress and novel stress phases, indicating that males are more vulnerable than females overall to display depression-like behavior immediately after a stressor and when exposed to a new stressor after a period of time. In particular, SD male rats in the sleep disruption condition displayed significantly more immobility than control

animals during the novel stress phase, indicating that male rats of this strain are particularly sensitive to the stressful, long-term effects of sleep disruption.

The fourth and final major finding of this study is with regard to voluntary alcohol consumption, a face-valid measure of stress effects. Interestingly, SD rats consumed greater total amounts of alcohol than LE rats. This finding is particularly remarkable, given that SD rats displayed greater depression-like behavior and greater anxiety-like behavior after a novel stressor. These results seem to indicate a particular vulnerability in SD rats. Another interesting finding is that **sleep disruption resulted in increased overall alcohol consumption**, a particularly compelling finding as sleep has been shown in this research to be a major independent factor in physiological markers of stress response (corticosterone), as well as depression-like behavior, and at least a partial factor in the expression of anxiety. Male rats in the sleep disruption condition consumed significantly more alcohol than did male rats in other conditions, and female rats showed no difference in alcohol consumption. The finding indicates a greater vulnerability for alcohol consumption in response to stress effects in male animals. Taken with the greater depression-like behavior displayed by male rats, the results of this research suggest a greater risk for depression-like behavior and alcohol abuse in adult males previously exposed to acute and chronic stressors during adolescence.

It is noteworthy that findings which occurred during the adult phase occurred several weeks after the conclusion of the stressors utilized. Even more interesting is that the restraint stress utilized during the novel stress period was a

totally new stressor and was initiated nearly 1 month after animals had been originally stressed. Still there were lingering effects of stress in adulthood based on stress history as an adolescent.

There are two findings of this research which are most important and quite concerning to not only military populations, but to other professions as well. First, a relatively minor sleep disruption administered chronically was either solely or partly responsible for depression-like behavior, anxiety-like behavior, and alcohol consumption. Given that sleep disruption and deprivation has been found in several studies to result in reduced stress tolerance, increased errors, increased accidents, poor decision-making, impaired memory, cognitive inefficiency, mood disorders, and anxiety disorders, it is not surprising that sleep was implicated in the current work as a meaningful stressor (Breslau, 1996; Halverson et al., 1995; Larsen, 2001; Lieberman et al., 2005; Van Dongen et al., 2004). In the most recent Mental Health Advisory Team (MHAT-V) report, researchers identified sleep as a major risk factor for mental health disorders and provided recommendations to address sleep problems in soldiers, because sleep is a modifiable and manageable behavior (MHAT 2008). Given the wealth of information available regarding the consequences of poor sleep, it is perplexing that, according to the MHAT-V report, officers underestimate the extent to which sleep has a negative impact on soldier performance.

What is most surprising and somewhat concerning is the second finding of this work—that the effects of stress in general and sleep in particular persisted well into adulthood and produced differential effects based on stress history. The

animals in this research were stressed for 14 days and then allowed to rest without stress manipulation for 14 days. Based on the estimated life span of approximately 2 years in rats, 14 days represents about 1.92% of their life span. Human life span is approximately 78 years (NCHS, 2009) or 39 times that of rats. 1.92% of 78 years represents about 1.5 years. Given the research model used in this work, the data suggest that the chronic effects of sleep disruption could last for years, even in the absence of additional stressors for a considerable period of time. The effect on military populations then is two-fold. Soldiers are exposed to the "primary" risk during the period of sleep disruption, but are then possibly subjected to increased risk of sleep-related problems long after they have returned to a more normal routine. Because the enemy's or "predator's" behavior is somewhat unpredictable and unmanageable, sleep is a logical behavior to target for intervention, because it is a modifiable behavior with far-reaching effects. The cost of inaction could result not only in ineffective unit members, but also veterans with increased disability risk and lower quality of life. The results of this work suggest that sleep management and hygiene should be of high priority from the outset, because it is unclear how the detrimental effects of sleep disruption progress over longer periods of time or if they remit.

LIMITATIONS AND FUTURE DIRECTIONS

Whereas this research offers an opportunity to make longitudinal observations over the developmental life-span, there are several limitations inherent in conducting research of this sort. For example, animals were pair-housed for logistical purposes and to provide an environment that was non-

stressful as compared to individual housing (Brown & Grunberg, 1995; Lawlor, 2002; Weiss et al., 2004; Zammit et al., 2001). Pair-housing also modeled the Army “battle buddy system,” wherein soldiers in training are placed in pairs to facilitate social support, assistance and teamwork (TRADOC, 2007). Although pair housing likely reduced stressful effects secondary to isolated housing, the result was decreased power and sensitivity with regard to measurements of food, water, and alcohol consumption, because the amount consumed was averaged between the two animals in each cage. True individual differences could not be assessed and the potential sample size was reduced to half, possibly masking an effect of food, water, or alcohol consumption.

Another limiting factor was the single measurement of corticosterone at the conclusion of the experiment. Tail vein puncture allows for a more frequent, non-lethal method of blood collection; however, this technique is invasive and requires any of a number of restraint techniques that could be stressful to the animals (Hem et al., 1998). Stress besides that which is part of the research methods could introduce confounding variables and jeopardize potential findings. Investigators replicating this study or conducing research in which assessment of stress hormones over a period of time is important, might consider fecal samples (Royo et al., 2004) as a non-invasive, non stressful method for collecting corticosterone samples.

Future experiments should consider the effects of stress condition on corticosterone at several time points throughout the experiment. Other militarily relevant stressors should be designed and incorporated to establish a better

model of distress faced by military deployed personnel. Based on the findings of this study, small-scale human experiments should be considered to determine if there are similar phenomenon that are occurring which might require more intensive investigation, analysis, and intervention.

POTENTIAL CLINICAL IMPLICATIONS

The clinical implications of this research are profound. In this research, sleep has emerged as a critical factor in various biological and behavioral markers of stress. The impact of sleep deprivation on military personnel and performance has been well documented (Lieberman et al., 2005; Belenky, 1997; Giam, 1997), **but the current research suggests that minimally disrupted sleep during late adolescence can have detrimental effects leading to anxiety, depression, and increased alcohol use later in adulthood**. Physiologically, sleep disruption might result in a greater stress response than even the threat of harm. If generalizable to humans, these findings could have major implications on work/rest cycles, sleeping environments in the combat theater, medication management, and a host of other issues related to sleep quality. Given the results of the current work, it might be advisable to employ sleep management and hygiene as the focal point of stress prevention efforts, prior to other interventions. In the MHAT-V report, recommendations are made with regard to managing and improving sleep within the forces. The report recommendations are made based on sleep disruption in close proximity to deployment, but the current findings raise the question how sleep disruption might have adverse effects, even after a substantial amount of recovery time.

These findings also have implications for management of individual patients and for understanding the etiology of their current distress. If poor quality sleep early in life adversely affects behavioral health later in life, then the manner in which psychosocial distress is currently viewed and assessed might require re-evaluation, particularly in a population with extensive deployment experience.

Another interesting, clinically relevant, and somewhat surprising finding was that females displayed more anxiety, while males displayed more depression-like behavior. These findings suggest that providers should be on the lookout for patients with deployment histories and understand that convention may not apply, depending on the individual's stress history. If the results of this research hold for humans, then not only are men more likely to be depressed than are women, they also are more likely to consume alcoholic substances. Whereas greater alcohol consumption in men might not be surprising, the possibility that men might be more depressed than women is unexpected according to current criteria and rates of depression (Kessler et al., 2005). It is notable, however, that although depression is more diagnosed in women, it remains unclear if depression actually occurs more frequently in women or if women are simply more likely than men to acknowledge depression and seek help (NIMH, 2005).

Strain differences are associated with underlying genetic, phenotypic variance. If anything can be hypothesized regarding genetic differences, then it is that genetics matter in the expression of stress effects. For example, SD rats were more sensitive to effects on depression-like behavior, regardless of time or

condition. Having a similar understanding in humans is vital to understanding where diagnostic and treatment starting points begin. There are also cumulative factors which might increase risk, such as in the case of SD male rats in the sleep disruption condition exhibiting more depression-like behavior than all other animals and consuming more alcohol.

There is much to be learned from this research with regard to the importance of adolescent stress history, gender, and possibly genotype and how they might affect the expression of stress effects in individuals.

CONCLUSIONS

In summary, sleep disruption, predator stress, and combined stress were useful manipulations to examine the effects of stress during adolescence on subsequent indices of behavioral health during adulthood in rodents. There were differences observed based on sex, strain, and the condition to which animals were assigned. Sleep disruption experienced during adolescence, although less than 10 minutes per hour during the low activity period for two weeks, resulted in noteworthy effects on serum corticosterone, depression-like behavior, alcohol consumption, and possibly anxiety-like behavior. There also were gender differences with previously stressed female rats being more likely to display anxiety-like behavior and previously stressed males more likely to display depression-like behavior. If applicable to the human condition, then it can be concluded that sleep hygiene should be considered as an important issue for mental/behavioral health enhancement efforts in young adults, not only in an immediate sense, but over a long period. Future research should focus on the full

extent to which sleep disruption has lasting effects and how those effects might be mitigated or eliminated.

APPENDIX A: LIST OF TABLES

Table 1. (Experiment 1 Timeline)

	Exp. Day	Age in days	Procedure	Stressor (urine + ____)
Baseline	1	22	Arrival, group assignment	*The listed stressors were administered at 3 and 7 minutes during the 10 minute stress period*
	2	23	Gentling	
	3	24	Gentling, BW	
	4	25	Gentling	
	5	26	Open field acclimation, BW	
	6	27	Baseline Open field	
Stress	7	28	Stress day 1, BW, FC, WC	Urine only
	8	29	Stress day 2	Alarm Bell/clap
	9	30	Stress day 3, BW, FC, WC	Bicycle bell
	10	31	Stress day 4	Whistle
	11	32	Stress day 5, BW, FC, WC	Cage shaking
	12	33	Stress day 6	Coins
	13	34	Stress day 7, BW, FC, WC	Shaking/clap
	14	35	Stress day 8	Lights/clapping
	15	36	Stress day 9, BW, FC, WC	Lights/coins
	16	37	Stress day 10	Whistle
	17	38	Stress day 11, BW, FC, WC	Lights/clapping
	18	39	Stress day 12	Coins
	19	40	Stress day 13, BW, FC, WC	Whistle
	20	41	Stress day 14	Cage shaking
Post-stress	21	42	Rest day 1, Open field, BW, FC, WC	
	22-34	43-56	Rest, BW, FC, WC (alternating days)	
Adult Phase	35	57	Open field, BW, FC, WC	
	36	58	Forced Swim Day 1	
	37	59	Forced Swim Day 2, BW, FC, WC	
	38	60	EtOH 3%	
	39	61	EtOH 3%, BW, FC, WC, AC	
	40	62	EtOH 3%, AC	
	41	63	EtOH, 6%, BW, FC, WC, AC	
	42	64	EtOH, 6%, AC	
	43	65	EtOH, 6%, BW, FC, WC, AC	
	44	66	EtOH, 12%	
	45	67	EtOH, 12%, BW, FC, WC, AC	
	46	68	EtOH, 12%, AC	
	47	69	EtOH 12%, AC	
	48	70	Idle	
	49	71	Sacrifice	

Table 2. Experiment 1 Forced Swim Test Rater Form

Date _____ Rater _____ Animal # _____

Time	Escape	Immobile	Swimming
1	Esc	Imm	Swm
2	Esc	Imm	Swm
3	Esc	Imm	Swm
4	Esc	Imm	Swm
5	Esc	Imm	Swm
6	Esc	Imm	Swm
7	Esc	Imm	Swm
8	Esc	Imm	Swm
9	Esc	Imm	Swm
10	Esc	Imm	Swm
11	Esc	Imm	Swm
12	Esc	Imm	Swm
13	Esc	Imm	Swm
14	Esc	Imm	Swm
15	Esc	Imm	Swm
16	Esc	Imm	Swm
17	Esc	Imm	Swm
18	Esc	Imm	Swm
19	Esc	Imm	Swm
20	Esc	Imm	Swm
21	Esc	Imm	Swm
22	Esc	Imm	Swm
23	Esc	Imm	Swm
24	Esc	Imm	Swm
25	Esc	Imm	Swm
26	Esc	Imm	Swm
27	Esc	Imm	Swm
28	Esc	Imm	Swm
29	Esc	Imm	Swm
30	Esc	Imm	Swm

Time	Escape	Immobile	Swimming
31	Esc	Imm	Swm
32	Esc	Imm	Swm
33	Esc	Imm	Swm
34	Esc	Imm	Swm
35	Esc	Imm	Swm
36	Esc	Imm	Swm
37	Esc	Imm	Swm
38	Esc	Imm	Swm
39	Esc	Imm	Swm
40	Esc	Imm	Swm
41	Esc	Imm	Swm
42	Esc	Imm	Swm
43	Esc	Imm	Swm
44	Esc	Imm	Swm
45	Esc	Imm	Swm
46	Esc	Imm	Swm
47	Esc	Imm	Swm
48	Esc	Imm	Swm
49	Esc	Imm	Swm
50	Esc	Imm	Swm
51	Esc	Imm	Swm
52	Esc	Imm	Swm
53	Esc	Imm	Swm
54	Esc	Imm	Swm
55	Esc	Imm	Swm
56	Esc	Imm	Swm
57	Esc	Imm	Swm
58	Esc	Imm	Swm
59	Esc	Imm	Swm
60	Esc	Imm	Swm

Table 3. Experiment 2 Timeline

	Exp. Day	Age	Procedure	DVs	Stressor
Baseline	1	25	Arrival, group assignment		*The listed stressors were administered at 3 and 7 minutes during the 10 minute stress period*
	2	26	Gentling		
	3	27	Gentling, BW	BW	
	4	28	Gentling	OF Acc, BW	
	5	29	Open field acclimation, BW	OF, BW	
	6	30	Baseline Open field	OF	
Stress	7	31	Stress day 1, BW, FC, WC	OF Acc	Urine only
	8	32	Stress day 2	OF	Alarm Bell/clap
	9	33	Stress day 3, BW, FC, WC		Bicycle bell
	10	34	Stress day 4	BW, FC	Whistle
	11	35	Stress day 5, BW, FC, WC	BW, FC	Cage shaking
	12	36	Stress day 6		Coins
	13	37	Stress day 7, BW, FC, WC		Shaking/clap
	14	38	Stress day 8		Lights/clapping
	15	39	Stress day 9, BW, FC, WC		Lights/coins
	16	40	Stress day 10		Whistle
	17	41	Stress day 11	BW, FC	Lights/clapping
	18	42	Stress day 12	BW, FC	Coins
	19	43	Stress day 13, BW,		Whistle
	20	44	Stress day 14		Cage shaking
Post-Stress	21	45	Rest day 1, Open field	OF	
	22	46	Forced Swim (Baseline)	FST D1	
	23	47	Forced Swim (Test Day 1)	FST D2	
	24-35	48-60	Rest, BW, FC (Thurs & Fridays)	BW, FC	
Adult Phase	36	61	Open field	OF	
	37	62	Forced Swim (Test Day 2)	FST	
	38	63	BW, FC, WC	EtOH, BW, FC	
	39	64	EtOH 3%	EtOH, BW, FC, WC	
	40	65	EtOH 3%, BW, FC, WC, AC	EtOH, WC	
	41	66	EtOH 3%, AC	EtOH, WC	
	42	67	EtOH, 6%, BW, FC, WC, AC	EtOH, WC	
	43	68	EtOH, 6%, AC	EtOH, WC	
	44	69	EtOH, 6%, BW, FC, WC, AC	EtOH, WC	
	45	70	EtOH, 12%	EtOH, WC	
	46	71	EtOH, 12%, BW, FC, WC, AC	EtOH, WC	
Novel Stress Phase	47	70	Restraint Stress/Open Field	OF, EtOH, WC	
	48	73	Restraint Stress/Forced Swim	PST, EtOH, WC	
	49	74	EtOH 3%	EtOH, WC, OF	
	50	75	EtOH 6%	PST	
	51	76	EtOH 12%	EtOH, WC	
	52	77	BW, FC, SAC	BW, FC, EtOH, WC	

EXPERIMENT 1 STATISCAL TABLES

Table 4. ANOVA - Corticosterone, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Condition	548670.587	3	182890.196	26.419	.000
Error	249218.550	36	6922.738		

Table 5. ANOVA Post Hoc Analysis (CORT), Experiment 1

(I) Stress Condition	(J) Stress Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Pred Stress	112.1202*	37.20951	.023	11.9065	212.3339
	Sleep Stress	-62.9959	37.20951	.342	-163.2096	37.2178
	Combined	-212.0460*	37.20951	.000	-312.2597	-111.8323
Pred Stress	Control	-112.1202*	37.20951	.023	-212.3339	-11.9065
	Sleep Stress	-175.1161*	37.20951	.000	-275.3298	-74.9024
	Combined	-324.1662*	37.20951	.000	-424.3799	-223.9525
Sleep Stress	Control	62.9959	37.20951	.342	-37.2178	163.2096
	Pred Stress	175.1161*	37.20951	.000	74.9024	275.3298
	Combined	-149.0501*	37.20951	.002	-249.2638	-48.8364
Combined	Control	212.0460*	37.20951	.000	111.8323	312.2597
	Pred Stress	324.1662*	37.20951	.000	223.9525	424.3799
	Sleep Stress	149.0501*	37.20951	.002	48.8364	249.2638

Table 6. Descriptive Statistics - Body Weight by Condition, Experiment 1

Condition		N	Minimum	Maximum	Mean	
		Statistic	Statistic	Statistic	Statistic	Std. Error
Baseline	BW DAY 2	10	40.30	59.30	49.5700	2.22860
	BW DAY 7	10	68.00	95.40	82.8010	3.37261
	BW DAY 13	10	109.20	150.20	131.9100	5.20663
	BW DAY 19	10	156.10	209.80	184.5200	6.29592

	BW DAY 25	10	201.00	262.90	233.2100	7.16474
	BW DAY 31	10	249.69	314.88	282.9390	8.05030
	BW DAY 37	10	291.07	362.28	328.3560	8.71990
	BW DAY 43	10	320.57	408.07	364.2920	9.39647
	BW DAY 49	10	352.88	446.71	401.2640	9.13837
	Valid N (listwise)	10				
Pred Stress	BW DAY 2	10	40.20	55.20	50.1000	1.68734
	BW DAY 7	10	72.30	92.40	82.5600	2.31983
	BW DAY 13	10	119.00	151.90	132.8600	3.15645
	BW DAY 19	10	172.70	209.00	184.5100	3.68554
	BW DAY 25	10	217.30	270.50	234.0700	5.32069
	BW DAY 31	10	265.02	339.55	289.4600	7.26195
	BW DAY 37	10	301.41	403.65	336.8610	10.13264
	BW DAY 43	10	339.11	452.77	376.3650	11.61817
	BW DAY 49	10	366.32	515.02	413.4650	14.16134
	Valid N (listwise)	10				
Sleep Disruption	BW DAY 2	10	34.90	58.00	48.7800	2.56623
	BW DAY 7	10	59.30	89.00	80.2600	3.38655
	BW DAY 13	10	96.00	142.80	126.9600	4.73350
	BW DAY 19	10	146.20	199.90	178.1100	5.36436
	BW DAY 25	10	190.10	254.90	225.0400	6.23202
	BW DAY 31	10	238.50	305.00	277.8600	6.91471
	BW DAY 37	10	285.60	357.70	319.6700	7.29499
	BW DAY 43	10	332.20	423.40	365.5700	8.06952
	BW DAY 49	10	364.10	469.10	401.4000	10.02030
	Valid N (listwise)	10				
Combined	BW DAY 2	10	41.20	56.50	48.4200	1.94003
	BW DAY 7	10	70.20	91.40	79.6800	2.74468
	BW DAY 13	10	111.20	148.10	130.1100	4.39872
	BW DAY 19	10	143.50	200.70	176.6800	6.09659
	BW DAY 25	10	182.40	257.60	226.4100	7.78847
	BW DAY 31	10	221.00	327.20	278.8500	10.18969

	BW DAY 37	10	255.80	390.10	326.5900	12.73243
	BW DAY 43	10	287.80	435.90	369.9800	13.94825
	BW DAY 49	10	305.80	472.70	405.2700	15.71985
	Valid N (listwise)	10				

Table 7. Repeated-Measures ANOVA (Body Weight) - Tests of Between-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Condition	2831.397	3	943.799	.259	.854	.021	.095
Error	131020.412	36	3639.456				

Table 8. Descriptive Statistics - Food Consumption by Condition, Experiment 1

Condition		N	Minimum	Maximum	Mean	
		Statistic	Statistic	Statistic	Statistic	Std. Error
Baseline	FC_Day7	5	51.12	64.51	58.8280	2.26875
	FC_Day13	5	76.79	99.19	85.3660	3.81343
	FC_Day19	5	100.34	122.06	107.8180	3.88831
	FC_Day25	5	106.62	126.08	114.5540	3.36726
	FC_Day31	5	122.70	148.86	134.1160	4.36288
	FC_Day37	5	96.18	119.26	108.3680	4.70912
	FC_Day43	5	97.01	116.57	107.7940	4.10540
	Valid N (listwise)	5				
Pred Stress	FC_Day7	5	58.99	106.71	70.4860	9.07839
	FC_Day13	5	68.76	87.06	79.1800	3.38114
	FC_Day19	5	43.84	109.14	91.6820	12.06389
	FC_Day25	5	114.65	125.77	120.9660	2.22190
	FC_Day31	5	126.22	147.17	136.0420	4.04026
	FC_Day37	5	113.35	169.19	128.6880	10.44875
	FC_Day43	5	104.04	123.48	112.6860	3.55856
	Valid N (listwise)	5				

Sleep Disruption	FC_Day7	5	48.20	62.80	55.6200	2.34785
	FC_Day13	5	70.00	87.20	79.3600	2.75038
	FC_Day19	5	89.20	107.60	99.6200	3.03272
	FC_Day25	5	105.30	119.30	111.9000	2.62736
	FC_Day31	5	118.70	132.10	126.3200	2.32710
	FC_Day37	5	111.50	125.50	115.6200	2.61714
	FC_Day43	5	96.20	120.80	112.6000	4.32065
	Valid N (listwise)	5				
Combined	FC_Day7	5	49.70	65.80	55.8800	2.68466
	FC_Day13	5	61.60	180.90	96.4800	21.63071
	FC_Day19	5	88.50	172.50	111.2200	15.71507
	FC_Day25	5	98.40	134.60	112.6200	6.32071
	FC_Day31	5	114.40	160.80	136.6000	7.85627
	FC_Day37	5	105.00	133.00	116.2800	4.71650
	FC_Day43	5	103.00	126.00	115.4400	3.70764
	Valid N (listwise)	5				

Table 9. Repeated-Measures ANOVA (Food Consumption) - Tests of Within-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Day	38618.293	5	7723.659	19.715	.000	.552	1.000
Day * Condition	7803.231	15	520.215	1.328	.206	.199	.753
Error(Day)	31341.424	80	391.768				

Table 10. Repeated-Measures ANOVA (Food Consumption) - Tests of Between-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Condition	5390.281	3	1796.760	1.763	.195	.248	.372
Error	16310.851	16	1019.428				

Table 11. Descriptive Statistics – Open Field Locomotion (Center Time), Experiment 1

	Condition	Mean	Std. Deviation	N
Ctr Time (BL)	Baseline	176.5400	50.62929	10
	Pred Stress	194.9200	126.06405	10
	Sleep Disruption	160.0200	112.59977	10
	Combined	129.7900	44.79793	10
	Total	165.3175	90.75107	40
Ctr Time (Post Stress)	Baseline	472.4400	295.50488	10
	Pred Stress	901.1200	293.67889	10
	Sleep Disruption	492.2600	301.84835	10
	Combined	595.2100	250.44423	10
	Total	615.2575	325.14657	40
Ctr Time (Adult)	Baseline	875.4300	481.47039	10
	Pred Stress	1033.2500	247.38041	10
	Sleep Disruption	573.2100	143.32662	10
	Combined	781.5500	338.92262	10
	Total	815.8600	356.78151	40

Table 12. Multivariate ANOVA (Center Time) - Tests of Between-Subjects Effects, Experiment 1

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Condition	Ctr Time (BL)	22925.193	3	7641.731	.922	.440	.071	.232
	Ctr Time (Post Stress)	1176444.945	3	392148.315	4.791	.007	.285	.867
	Ctr Time (Adult)	1108631.956	3	369543.985	3.450	.027	.223	.726
Error	Ctr Time (BL)	298269.285	36	8285.258				
	Ctr Time (Post Stress)	2946646.473	36	81851.291				
	Ctr Time (Adult)	3855796.780	36	107105.466				

Table 13. Post Hoc Analysis (Center Time), Experiment 1

Dependent Variable	(I) Stress Condition	(J) Stress Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Ctr Time (BL)	No Stress	Pred Stress	-18.3800	40.70690	.969	-128.0129	91.2529
		Sleep Stress	16.5200	40.70690	.977	-93.1129	126.1529
		Combined	46.7500	40.70690	.662	-62.8829	156.3829
	Pred Stress	No Stress	18.3800	40.70690	.969	-91.2529	128.0129
		Sleep Stress	34.9000	40.70690	.827	-74.7329	144.5329
		Combined	65.1300	40.70690	.391	-44.5029	174.7629
	Sleep Stress	No Stress	-16.5200	40.70690	.977	-126.1529	93.1129
		Pred Stress	-34.9000	40.70690	.827	-144.5329	74.7329
		Combined	30.2300	40.70690	.879	-79.4029	139.8629
	Combined	No Stress	-46.7500	40.70690	.662	-156.3829	62.8829
		Pred Stress	-65.1300	40.70690	.391	-174.7629	44.5029
		Sleep Stress	-30.2300	40.70690	.879	-139.8629	79.4029

Ctr Time (Post Stress)	No Stress	Pred Stress	-428.6800*	127.94631	.010	-773.2685	-84.0915
		Sleep Stress	-19.8200	127.94631	.999	-364.4085	324.7685
		Combined	-122.7700	127.94631	.773	-467.3585	221.8185
	Pred Stress	No Stress	428.6800*	127.94631	.010	84.0915	773.2685
		Sleep Stress	408.8600*	127.94631	.015	64.2715	753.4485
		Combined	305.9100	127.94631	.097	-38.6785	650.4985
	Sleep Stress	No Stress	19.8200	127.94631	.999	-324.7685	364.4085
		Pred Stress	-408.8600*	127.94631	.015	-753.4485	-64.2715
		Combined	-102.9500	127.94631	.852	-447.5385	241.6385
	Combined	No Stress	122.7700	127.94631	.773	-221.8185	467.3585
		Pred Stress	-305.9100	127.94631	.097	-650.4985	38.6785
		Sleep Stress	102.9500	127.94631	.852	-241.6385	447.5385
Ctr Time (Adult)	No Stress	Pred Stress	-157.8200	146.35947	.705	-551.9993	236.3593
		Sleep Stress	302.2200	146.35947	.184	-91.9593	696.3993
		Combined	93.8800	146.35947	.918	-300.2993	488.0593
	Pred Stress	No Stress	157.8200	146.35947	.705	-236.3593	551.9993
		Sleep Stress	460.0400*	146.35947	.017	65.8607	854.2193
		Combined	251.7000	146.35947	.329	-142.4793	645.8793
	Sleep Stress	No Stress	-302.2200	146.35947	.184	-696.3993	91.9593
		Pred Stress	-460.0400*	146.35947	.017	-854.2193	-65.8607
		Combined	-208.3400	146.35947	.493	-602.5193	185.8393
	Combined	No Stress	-93.8800	146.35947	.918	-488.0593	300.2993
		Pred Stress	-251.7000	146.35947	.329	-645.8793	142.4793
		Sleep Stress	208.3400	146.35947	.493	-185.8393	602.5193

Table 14. Horizontal Activity – Descriptive Statistics, Experiment 1

	Stress Condition	Mean	Std. Deviation	N
Horz Act (BL)	No Stress	8223.5000	1493.50998	10
	Pred Stress	8814.9000	2414.58266	10
	Sleep Stress	7511.7000	1937.47476	10
	Combined	6605.3000	1368.36326	10
	Total	7788.8500	1963.61840	40

Horz Act (Post Stress)	No Stress	14498.6000	4737.77677	10
	Pred Stress	19934.6000	2929.10362	10
	Sleep Stress	14052.2000	2893.12614	10
	Combined	18412.5000	3842.47326	10
	Total	16724.4750	4355.80264	40
Horz Act (Adult)	No Stress	16755.5000	5066.79834	10
	Pred Stress	20268.0000	4754.86065	10
	Sleep Stress	14595.3000	4727.01233	10
	Combined	18242.8000	5107.06373	10
	Total	17465.4000	5169.86959	40

Table 15. Horizontal Activity Repeated-Measures ANOVA – Tests of Within-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Observed Power
Phase	2.320E9	2	1.160E9	120.171	.000	1.000
Phase * Condition	1.473E8	6	2.455E7	2.543	.027	.813
Error(Phase)	6.951E8	72	9654591.126			

Table 16. Horizontal Activity Repeated-Measures ANOVA – Tests of Between-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Condition	3.044E8	3	1.015E8	4.648	.008	.279	.856
Error	7.859E8	36	2.183E7				

Table 17. Horizontal Activity Repeated-Measures ANOVA – Post Hoc Analysis, Experiment 1

(I) Stress Condition	(J) Stress Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
No Stress	Pred Stress	-3179.9667	1206.36593	.057	-6428.9842	69.0508
	Sleep Stress	1106.1333	1206.36593	.796	-2142.8842	4355.1508
	Combined	-1261.0000	1206.36593	.724	-4510.0175	1988.0175
Pred Stress	No Stress	3179.9667	1206.36593	.057	-69.0508	6428.9842
	Sleep Stress	4286.1000*	1206.36593	.006	1037.0825	7535.1175
	Combined	1918.9667	1206.36593	.397	-1330.0508	5167.9842
Sleep Stress	No Stress	-1106.1333	1206.36593	.796	-4355.1508	2142.8842
	Pred Stress	-4286.1000*	1206.36593	.006	-7535.1175	-1037.0825
	Combined	-2367.1333	1206.36593	.221	-5616.1508	881.8842
Combined	No Stress	1261.0000	1206.36593	.724	-1988.0175	4510.0175
	Pred Stress	-1918.9667	1206.36593	.397	-5167.9842	1330.0508
	Sleep Stress	2367.1333	1206.36593	.221	-881.8842	5616.1508

Table 18. Center Time / Horizontal Activity Ratio - Descriptive Statistics, Experiment 1

	Stress Condition	Mean	Std. Deviation	N
CTRTME_Horz_Ratio_BL	No Stress	.0216	.00521	10
	Pred Stress	.0212	.00838	10
	Sleep Stress	.0199	.00868	10
	Combined	.0199	.00698	10
	Total	.0206	.00719	40
CTRTME_Horz_Ratio_PS	No Stress	.0306	.01005	10
	Pred Stress	.0461	.01721	10
	Sleep Stress	.0336	.01539	10
	Combined	.0333	.01509	10
	Total	.0359	.01536	40

CTRTME_Horz_Ratio_Adult	No Stress	.0574	.04197	10
	Pred Stress	.0520	.01167	10
	Sleep Stress	.0405	.00894	10
	Combined	.0434	.01703	10
	Total	.0483	.02387	40

Table 19. Center Time / Horizontal Activity Ratio, Repeated-Measures ANOVA – Tests of Within-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Phase	.015	2	.008	27.301	.000	.431	1.000
Phase * Condition	.002	6	.000	1.120	.360	.085	.414
Error(Phase)	.020	72	.000				

Table 20. Center Time / Horz Activity Ratio, Repeated-Measures ANOVA – Tests of Between-Subjects Effects, Experiment 1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Condition	.001	3	.000	1.691	.186	.124	.405
Error	.010	36	.000				

Table 21. Descriptives - Forced Swim Immobility, Experiment 1

Immobility			
No Stress	N	Valid	10
		Median	24
Pred Stress	N	Valid	10
		Median	20
Sleep Stress	N	Valid	10
		Median	17
Combined	N	Valid	10
		Median	22

Table 22. Chi Square (Forced Swim Test), Experiment 1

	Immobility
N	40
Median	22.0000
Chi-Square	.404
df	3
Asymp. Sig.	.939

Table 23. Descriptive Statistics - Ethanol Consumption, Experiment 1

	Condition	Mean	Std. Deviation	N
Total 3%	Baseline	91.5220	43.66590	5
	Pred Stress	125.8380	63.04104	5
	Sleep Disruption	106.8000	54.47784	5
	Combined	106.4400	61.41554	5
	Total	107.6500	53.03815	20
Total 6%	Baseline	71.5640	44.73881	5
	Pred Stress	61.4160	37.39946	5
	Sleep Disruption	54.8200	26.16920	5
	Combined	72.9800	51.59769	5
	Total	65.1950	38.46063	20
Total 12%	Baseline	28.1260	16.71719	5
	Pred Stress	29.4460	21.25566	5
	Sleep Disruption	17.8200	5.39092	5
	Combined	27.9200	17.82868	5
	Total	25.8280	15.80663	20
Tot_EtOH	Baseline	191.2120	98.83916	5
	Pred Stress	216.7000	82.89483	5
	Sleep Disruption	179.4400	76.91000	5
	Combined	207.3400	124.50853	5
	Total	198.6730	90.71683	20

Table 24. Multivariate ANOVA (Ethanol Consumption) –
Tests of Between-Subjects Effects, Experiment 1

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Condition	Total 3%	2965.512	3	988.504	.313	.816	.055	.098
	Total 6%	1115.459	3	371.820	.220	.881	.040	.083
	Total 12%	434.376	3	144.792	.537	.664	.092	.136
	Tot_EtOH	4128.322	3	1376.107	.145	.932	.026	.071
Error	Total 3%	50482.348	16	3155.147				
	Total 6%	26989.717	16	1686.857				
	Total 12%	4312.766	16	269.548				
	Tot_EtOH	152233.013	16	9514.563				

EXPERIMENT 2 STATISTICAL TABLES

Table 25. ANOVA – Corticosterone by Condition, Experiment 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1.014E6	15	67628.161	3.808	.000
Intercept	9743678.732	1	9743678.732	548.626	.000
SEX	376280.197	1	376280.197	21.187	.000
STRAIN	2943.361	1	2943.361	.166	.685
CONDITION	254342.393	3	84780.798	4.774	.003
SEX * STRAIN	12537.140	1	12537.140	.706	.402
SEX * CONDITION	157150.719	3	52383.573	2.950	.035
STRAIN * CONDITION	70553.355	3	23517.785	1.324	.269
SEX * STRAIN * CONDITION	146409.122	3	48803.041	2.748	.045
Error	2539699.331	143	17760.135		

Table 26. ANOVA – Corticosterone Post Hoc Analysis, Experiment 2

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Predator	-95.8345*	29.98986	.009	-173.7930	-17.8759
	Sleep	-95.5286*	29.98986	.010	-173.4872	-17.5700
	Combined	-79.6076*	29.98986	.043	-157.5662	-1.6491
Predator	Control	95.8345*	29.98986	.009	17.8759	173.7930
	Sleep	.3059	29.79944	1.000	-77.1577	77.7695
	Combined	16.2269	29.79944	.948	-61.2367	93.6904
Sleep	Control	95.5286*	29.98986	.010	17.5700	173.4872
	Predator	-.3059	29.79944	1.000	-77.7695	77.1577
	Combined	15.9210	29.79944	.951	-61.5426	93.3846
Combined	Control	79.6076*	29.98986	.043	1.6491	157.5662
	Predator	-16.2269	29.79944	.948	-93.6904	61.2367
	Sleep	-15.9210	29.79944	.951	-93.3846	61.5426

Table 27. CORT ANOVA, by Sex, Experiment 2

Females					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	247231.773[a]	3	82410.591	2.976	.037
Intercept	7023187.644	1	7023187.644	253.654	.000
CONDITION	247231.773	3	82410.591	2.976	.037
Error	2104290.822	76	27688.037		
Males					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	164620.762a	3	54873.587	6.153	.001
Intercept	3136692.117	1	3136692.117	351.701	.000
CONDITION	164620.762	3	54873.587	6.153	.001
Error	668898.166	75	8918.642		

Table 28. CORT Post Hoc Analysis (Females), Experiment 2

		Mean Difference			95% Confidence Interval	
(I) Condition	(J) Condition	(I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Control	Predator	-110.4743	52.61942	.163	-248.6948	27.7462
	Sleep	-87.1379	52.61942	.354	-225.3584	51.0826
	Combined	-152.1328*	52.61942	.025	-290.3534	-13.9123
Predator	Control	110.4743	52.61942	.163	-27.7462	248.6948
	Sleep	23.3364	52.61942	.971	-114.8841	161.5569
	Combined	-41.6585	52.61942	.858	-179.8791	96.5620
Sleep	Control	87.1379	52.61942	.354	-51.0826	225.3584
	Predator	-23.3364	52.61942	.971	-161.5569	114.8841
	Combined	-64.9949	52.61942	.607	-203.2155	73.2256
Combined	Control	152.1328*	52.61942	.025	13.9123	290.3534
	Predator	41.6585	52.61942	.858	-96.5620	179.8791
	Sleep	64.9949	52.61942	.607	-73.2256	203.2155

Table 29. CORT Post Hoc Analysis (Males), Experiment 2

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Predator	-82.7004*	30.25449	.038	-162.1965	-3.2043
	Sleep	-105.4251*	30.25449	.005	-184.9212	-25.9290
	Combined	-8.5882	30.25449	.992	-88.0843	70.9079
Predator	Control	82.7004*	30.25449	.038	3.2043	162.1965
	Sleep	-22.7246	29.86410	.872	-101.1949	55.7456
	Combined	74.1123	29.86410	.071	-4.3580	152.5825
Sleep	Control	105.4251*	30.25449	.005	25.9290	184.9212
	Predator	22.7246	29.86410	.872	-55.7456	101.1949
	Combined	96.8369*	29.86410	.009	18.3666	175.3072
Combined	Control	8.5882	30.25449	.992	-70.9079	88.0843
	Predator	-74.1123	29.86410	.071	-152.5825	4.3580
	Sleep	-96.8369*	29.86410	.009	-175.3072	-18.3666

Table 30. Descriptives - Weekly Body Weight, Experiment 2

	N	Minimum	Maximum	Mean	
	Statistic	Statistic	Statistic	Statistic	Std. Error
1st BW	160	60.50	99.73	78.1435	.78327
2nd BW	160	94.26	164.18	125.0220	1.03580
3rd BW	160	123.71	240.38	174.3375	1.83404
4th BW	160	148.74	296.80	216.0079	2.87569
5th BW	160	158.94	504.05	264.8411	4.65748
6th BW	160	180.02	433.00	297.0915	5.58378
7th BW	160	190.70	487.00	327.6350	6.51190
8th BW	160	202.13	524.80	345.9806	7.09482
Valid N (listwise)	160				

Table 31. Descriptives - Weekly Body Weight by Sex, Experiment 2

Sex		N	Mean	
		Statistic	Statistic	Std. Error
Male	1st BW	80	77.3561	.96852
	2nd BW	80	130.7242	1.37912
	3rd BW	80	192.8043	1.84112
	4th BW	80	247.7735	2.33112
	5th BW	80	316.9138	3.79119
	6th BW	80	362.2854	3.39985
	7th BW	80	403.8596	3.92496
	8th BW	80	428.4679	4.47706
	Valid N (listwise)	80		
Female	1st BW	80	78.9309	1.23120
	2nd BW	80	119.3198	1.26237
	3rd BW	80	155.8708	1.23161
	4th BW	80	184.2422	1.51915
	5th BW	80	212.7685	2.07299
	6th BW	80	231.8976	2.51866
	7th BW	80	251.4104	2.86253
	8th BW	80	263.4932	3.21233
	Valid N (listwise)	80		

Table 32. Descriptives - Weekly Body Weight by Sex and Strain, Experiment 2

Sex	Strain		N	Mean	
			Statistic	Statistic	Std. Error
Male	SD	1st BW	40	80.2653	1.29912
		2nd BW	40	134.4845	1.76636
		3rd BW	40	196.7485	2.26459
		4th BW	40	247.8378	2.86041

		5th BW	40	316.2290	6.09446
		6th BW	40	359.9103	4.48175
		7th BW	40	399.0105	5.18421
		8th BW	40	424.0030	5.99374
		Valid N (listwise)	40		
	LE	1st BW	40	74.4470	1.29556
		2nd BW	40	126.9640	1.96476
		3rd BW	40	188.8600	2.79403
		4th BW	40	247.7092	3.71926
		5th BW	40	317.5985	4.58945
		6th BW	40	364.6605	5.14329
		7th BW	40	408.7087	5.85914
		8th BW	40	432.9328	6.65258
		Valid N (listwise)	40		
Female	SD	1st BW	40	86.6105	1.37944
		2nd BW	40	125.4488	1.73541
		3rd BW	40	157.4180	1.93675
		4th BW	40	182.1670	2.23055
		5th BW	40	205.3250	2.76927
		6th BW	40	220.7325	3.12556
		7th BW	40	237.4063	3.37127
		8th BW	40	247.0685	3.37290
		Valid N (listwise)	40		
	LE	1st BW	40	71.2513	1.10176
		2nd BW	40	113.1908	1.23205
		3rd BW	40	154.3235	1.50714
		4th BW	40	186.3175	2.03800
		5th BW	40	220.2120	2.62668
		6th BW	40	243.0628	3.08836
		7th BW	40	265.4145	3.43129
		8th BW	40	279.9180	4.07364
		Valid N (listwise)	40		

Table 33. Descriptives - Body Weight by Stress Condition, Experiment 2

Condition		N	Mean	
		Statistic	Statistic	Std. Error
Control	1st BW	40	76.8875	1.46238
	2nd BW	40	124.6975	2.05949
	3rd BW	40	176.0025	3.72929
	4th BW	40	217.8425	5.88837
	5th BW	40	263.6700	8.91117
	6th BW	40	294.3050	11.24434
	7th BW	40	325.7775	13.18457
	8th BW	40	344.3625	14.64597
	Valid N (listwise)	40		
Predator	1st BW	40	78.8225	1.61730
	2nd BW	40	124.1600	2.00804
	3rd BW	40	172.3950	3.67381
	4th BW	40	213.3203	5.86284
	5th BW	40	265.7850	9.42629
	6th BW	40	300.4475	11.93730
	7th BW	40	331.2900	14.14066
	8th BW	40	352.9800	14.97348
	Valid N (listwise)	40		
Sleep	1st BW	40	79.0970	1.67259
	2nd BW	40	127.4515	2.25140
	3rd BW	40	177.8690	4.03052
	4th BW	40	219.9843	6.07867
	5th BW	40	264.9670	8.81854
	6th BW	40	299.6420	11.11262
	7th BW	40	327.6850	12.79524
	8th BW	40	346.4780	13.86533
	Valid N (listwise)	40		
Combined	1st BW	40	77.7670	1.54077

	2nd BW	40	123.7790	1.98523
	3rd BW	40	171.0835	3.23200
	4th BW	40	212.8845	5.28529
	5th BW	40	264.9425	10.37585
	6th BW	40	293.9715	10.72950
	7th BW	40	325.7875	12.38939
	8th BW	40	340.1018	13.70033
	Valid N (listwise)	40		

Table 34. Descriptives - Body Weight by Sex, Strain, and Condition, Experiment 2

	Sex	Strain	Condition	Mean	Std. Deviation	N
1st BW	Male	SD	Control	80.5700	8.35784	10
			Predator	78.6700	10.14167	10
			Sleep	82.3970	6.75544	10
			Combined	79.4240	8.08887	10
			Total	80.2653	8.21639	40
		LE	Control	72.8400	6.09028	10
			Predator	79.6100	7.59919	10
			Sleep	72.4230	8.24599	10
			Combined	72.9150	9.40490	10
			Total	74.4470	8.19383	40
		Total	Control	76.7050	8.14755	20
			Predator	79.1400	8.73537	20
			Sleep	77.4100	8.94455	20
			Combined	76.1695	9.16737	20
			Total	77.3561	8.66267	80
	Female	SD	Control	84.8500	7.55899	10
			Predator	87.4400	8.87996	10
			Sleep	87.4140	10.49615	10
			Combined	86.7380	8.87273	10
			Total	86.6105	8.72437	40

		LE	Control	69.2900	6.22494	10
			Predator	69.5700	5.96453	10
			Sleep	74.1540	9.77305	10
			Combined	71.9910	4.81466	10
			Total	71.2512	6.96812	40
		Total	Control	77.0700	10.44676	20
			Predator	78.5050	11.75753	20
			Sleep	80.7840	11.98743	20
			Combined	79.3645	10.27139	20
			Total	78.9309	11.01221	80
	Total	SD	Control	82.7100	8.06068	20
			Predator	83.0550	10.31077	20
			Sleep	84.9055	8.96806	20
			Combined	83.0810	9.07533	20
			Total	83.4379	9.00532	80
		LE	Control	71.0650	6.26429	20
			Predator	74.5900	8.41026	20
			Sleep	73.2885	8.84534	20
			Combined	72.4530	7.28722	20
			Total	72.8491	7.72658	80
		Total	Control	76.8875	9.24893	40
			Predator	78.8225	10.22868	40
			Sleep	79.0970	10.57841	40
			Combined	77.7670	9.74466	40
			Total	78.1435	9.90767	160
2nd BW	Male	SD	Control	136.2000	11.75623	10
			Predator	129.3900	10.37159	10
			Sleep	140.3160	11.42329	10
			Combined	132.0320	9.37868	10
			Total	134.4845	11.17145	40
		LE	Control	125.9600	8.79245	10
			Predator	133.1200	11.62448	10

			Sleep	125.7890	13.55898	10
			Combined	122.9870	14.50865	10
			Total	126.9640	12.42625	40
		Total	Control	131.0800	11.38774	20
			Predator	131.2550	10.89145	20
			Sleep	133.0525	14.29797	20
			Combined	127.5095	12.76344	20
			Total	130.7242	12.33523	80
	Female	SD	Control	125.2400	9.55745	10
			Predator	123.3600	8.46131	10
			Sleep	126.6050	14.40574	10
			Combined	126.5900	11.98865	10
			Total	125.4488	10.97571	40
		LE	Control	111.3900	9.08093	10
			Predator	110.7700	8.06888	10
			Sleep	117.0960	7.05864	10
			Combined	113.5070	6.20849	10
			Total	113.1907	7.79216	40
		Total	Control	118.3150	11.52431	20
			Predator	117.0650	10.31821	20
			Sleep	121.8505	12.07052	20
			Combined	120.0485	11.46225	20
			Total	119.3198	11.29095	80
	Total	SD	Control	130.7200	11.84681	20
			Predator	126.3750	9.71780	20
			Sleep	133.4605	14.47704	20
			Combined	129.3110	10.84160	20
			Total	129.9666	11.90592	80
		LE	Control	118.6750	11.46931	20
			Predator	121.9450	15.04332	20
			Sleep	121.4425	11.42683	20
			Combined	118.2470	11.90039	20

			Total	120.0774	12.41890	80
		Total	Control	124.6975	13.02539	40
			Predator	124.1600	12.69995	40
			Sleep	127.4515	14.23908	40
			Combined	123.7790	12.55567	40
			Total	125.0220	13.10200	160
3rd BW	Male	SD	Control	199.2800	14.74546	10
			Predator	189.6700	11.22814	10
			Sleep	206.5390	15.30589	10
			Combined	191.5050	10.54999	10
			Total	196.7485	14.32254	40
		LE	Control	192.6600	12.22067	10
			Predator	194.7400	16.29991	10
			Sleep	188.8160	18.94577	10
			Combined	179.2240	20.53130	10
			Total	188.8600	17.67097	40
		Total	Control	195.9700	13.61130	20
			Predator	192.2050	13.86846	20
			Sleep	197.6775	19.06973	20
			Combined	185.3645	17.09054	20
			Total	192.8043	16.46750	80
	Female	SD	Control	158.6200	10.53310	10
			Predator	153.4500	8.05581	10
			Sleep	159.3470	15.43071	10
			Combined	158.2550	14.56752	10
			Total	157.4180	12.24909	40
		LE	Control	153.4500	11.03321	10
			Predator	151.7200	11.07688	10
			Sleep	156.7740	7.90105	10
			Combined	155.3500	8.40505	10
			Total	154.3235	9.53200	40
		Total	Control	156.0350	10.82819	20

			Predator	152.5850	9.46824	20
			Sleep	158.0605	12.00417	20
			Combined	156.8025	11.67073	20
			Total	155.8708	11.01586	80
	Total	SD	Control	178.9500	24.30243	20
			Predator	171.5600	20.87324	20
			Sleep	182.9430	28.45756	20
			Combined	174.8800	21.07561	20
			Total	177.0833	23.81086	80
		LE	Control	173.0550	23.08658	20
			Predator	173.2300	25.90373	20
			Sleep	172.7950	21.67435	20
			Combined	167.2870	19.57368	20
			Total	171.5917	22.38251	80
		Total	Control	176.0025	23.58611	40
			Predator	172.3950	23.23520	40
			Sleep	177.8690	25.49128	40
			Combined	171.0835	20.44093	40
			Total	174.3375	23.19903	160
4th BW	Male	SD	Control	252.9600	19.98756	10
			Predator	236.1250	14.93822	10
			Sleep	256.7310	19.50284	10
			Combined	245.5350	11.82528	10
			Total	247.8378	18.09083	40
		LE	Control	250.0900	14.41353	10
			Predator	256.0540	21.02250	10
			Sleep	248.4330	28.35094	10
			Combined	236.2600	26.72697	10
			Total	247.7092	23.52268	40
		Total	Control	251.5250	17.02391	20
			Predator	246.0895	20.48323	20
			Sleep	252.5820	24.06298	20

			Combined	240.8975	20.66989	20
			Total	247.7735	20.85013	80
	Female	SD	Control	184.6800	13.30395	10
			Predator	177.3980	9.75157	10
			Sleep	185.3540	16.62687	10
			Combined	181.2360	16.41084	10
			Total	182.1670	14.10722	40
		LE	Control	183.6400	13.35225	10
			Predator	183.7040	13.50889	10
			Sleep	189.4190	13.18961	10
			Combined	188.5070	12.39455	10
			Total	186.3175	12.88947	40
		Total	Control	184.1600	12.98361	20
			Predator	180.5510	11.91433	20
			Sleep	187.3865	14.75482	20
			Combined	184.8715	14.63737	20
			Total	184.2423	13.58772	80
	Total	SD	Control	218.8200	38.72934	20
			Predator	206.7615	32.53217	20
			Sleep	221.0425	40.64267	20
			Combined	213.3855	35.80222	20
			Total	215.0024	36.76444	80
		LE	Control	216.8650	36.67231	20
			Predator	219.8790	40.90588	20
			Sleep	218.9260	37.14335	20
			Combined	212.3835	31.79982	20
			Total	217.0134	36.18477	80
		Total	Control	217.8425	37.24135	40
			Predator	213.3203	37.07986	40
			Sleep	219.9843	38.44491	40
			Combined	212.8845	33.42714	40
			Total	216.0079	36.37486	160

5th BW	Male	SD	Control	312.7900	26.15826	10
			Predator	305.3900	22.58064	10
			Sleep	318.2890	30.43004	10
			Combined	328.4470	63.27426	10
			Total	316.2290	38.54473	40
		LE	Control	319.0600	20.69268	10
			Predator	334.1100	23.08239	10
			Sleep	310.0630	30.48319	10
			Combined	307.1610	35.65519	10
			Total	317.5985	29.02625	40
		Total	Control	315.9250	23.17955	20
			Predator	319.7500	26.66392	20
			Sleep	314.1760	29.94314	20
			Combined	317.8040	51.16525	20
			Total	316.9138	33.90943	80
	Female	SD	Control	206.8700	15.30236	10
			Predator	199.5600	11.77023	10
			Sleep	209.8660	23.16758	10
			Combined	205.0040	18.90038	10
			Total	205.3250	17.51440	40
		LE	Control	215.9600	14.62693	10
			Predator	224.0800	22.01938	10
			Sleep	221.6500	14.50974	10
			Combined	219.1580	15.69471	10
			Total	220.2120	16.61256	40
		Total	Control	211.4150	15.29727	20
			Predator	211.8200	21.29575	20
			Sleep	215.7580	19.76139	20
			Combined	212.0810	18.40139	20
			Total	212.7685	18.54143	80
	Total	SD	Control	259.8300	58.20155	20
			Predator	252.4750	57.04834	20

			Sleep	264.0775	61.53401	20
			Combined	266.7255	77.94687	20
			Total	260.7770	63.23550	80
		LE	Control	267.5100	55.69053	20
			Predator	279.0950	60.56395	20
			Sleep	265.8565	50.96031	20
			Combined	263.1595	52.50623	20
			Total	268.9052	54.34351	80
		Total	Control	263.6700	56.35921	40
			Predator	265.7850	59.61712	40
			Sleep	264.9670	55.77335	40
			Combined	264.9425	65.62264	40
			Total	264.8411	58.91292	160
6th BW	Male	SD	Control	357.3900	31.09610	10
			Predator	355.2200	26.98015	10
			Sleep	367.1230	39.02922	10
			Combined	359.9080	13.19484	10
			Total	359.9102	28.34506	40
		LE	Control	364.3400	24.68905	10
			Predator	384.3000	26.23674	10
			Sleep	357.5850	35.18868	10
			Combined	352.4170	37.39293	10
			Total	364.6605	32.52900	40
		Total	Control	360.8650	27.55871	20
			Predator	369.7600	29.89005	20
			Sleep	362.3540	36.49699	20
			Combined	356.1625	27.56007	20
			Total	362.2854	30.40920	80
	Female	SD	Control	221.4500	17.19614	10
			Predator	213.9900	15.45427	10
			Sleep	227.5250	24.42476	10
			Combined	219.9650	21.42906	10

			Total	220.7325	19.76780	40
		LE	Control	234.0400	15.43914	10
			Predator	248.2800	23.86568	10
			Sleep	246.3350	17.90811	10
			Combined	243.5960	19.79137	10
			Total	243.0628	19.53251	40
		Total	Control	227.7450	17.16669	20
			Predator	231.1350	26.31256	20
			Sleep	236.9300	22.96966	20
			Combined	231.7805	23.45235	20
			Total	231.8976	22.52759	80
	Total	SD	Control	289.4200	73.89982	20
			Predator	284.6050	75.54379	20
			Sleep	297.3240	78.30999	20
			Combined	289.9365	73.84906	20
			Total	290.3214	74.11785	80
		LE	Control	299.1900	69.78225	20
			Predator	316.2900	73.92337	20
			Sleep	301.9600	63.20947	20
			Combined	298.0065	62.96173	20
			Total	303.8616	66.73837	80
		Total	Control	294.3050	71.11542	40
			Predator	300.4475	75.49808	40
			Sleep	299.6420	70.28237	40
			Combined	293.9715	67.85933	40
			Total	297.0915	70.62984	160
7th BW	Male	SD	Control	398.6100	34.99055	10
			Predator	396.9200	32.42060	10
			Sleep	403.2050	46.02843	10
			Combined	397.3070	15.38372	10
			Total	399.0105	32.78784	40
		LE	Control	408.8000	28.95663	10

			Predator	430.8900	30.41383	10
			Sleep	397.1820	40.23056	10
			Combined	397.9630	41.78335	10
			Total	408.7087	37.05645	40
		Total	Control	403.7050	31.69311	20
			Predator	413.9050	35.20965	20
			Sleep	400.1935	42.18719	20
			Combined	397.6350	30.64630	20
			Total	403.8596	35.10595	80
	Female	SD	Control	239.9800	21.04359	10
			Predator	228.7400	16.97418	10
			Sleep	244.8390	24.61019	10
			Combined	236.0660	21.89082	10
			Total	237.4062	21.32179	40
		LE	Control	255.7200	20.98332	10
			Predator	268.6100	23.16532	10
			Sleep	265.5140	20.26866	10
			Combined	271.8140	22.17544	10
			Total	265.4145	21.70140	40
		Total	Control	247.8500	21.98915	20
			Predator	248.6750	28.44281	20
			Sleep	255.1765	24.37168	20
			Combined	253.9400	28.21741	20
			Total	251.4104	25.60322	80
	Total	SD	Control	319.2950	86.09112	20
			Predator	312.8300	89.87580	20
			Sleep	324.0220	88.82788	20
			Combined	316.6865	84.73988	20
			Total	318.2084	85.82994	80
		LE	Control	332.2600	82.29488	20
			Predator	349.7500	87.30729	20
			Sleep	331.3480	74.32013	20

			Combined	334.8885	72.44099	20
			Total	337.0616	78.15806	80
		Total	Control	325.7775	83.38655	40
			Predator	331.2900	89.43341	40
			Sleep	327.6850	80.92420	40
			Combined	325.7875	78.35741	40
			Total	327.6350	82.36975	160
8th BW	Male	SD	Control	426.0100	42.16324	10
			Predator	421.6700	36.63760	10
			Sleep	428.1740	51.78228	10
			Combined	420.1580	19.47438	10
			Total	424.0030	37.90775	40
		LE	Control	434.7500	33.14374	10
			Predator	457.8900	35.94717	10
			Sleep	419.9870	45.95264	10
			Combined	419.1040	45.54559	10
			Total	432.9328	42.07463	40
		Total	Control	430.3800	37.18245	20
			Predator	439.7800	39.91442	20
			Sleep	424.0805	47.83336	20
			Combined	419.6310	34.09616	20
			Total	428.4679	40.04403	80
	Female	SD	Control	250.0800	22.67661	10
			Predator	243.3700	14.58836	10
			Sleep	254.5930	27.12338	10
			Combined	240.2310	19.21083	10
			Total	247.0685	21.33206	40
		LE	Control	266.6100	26.67518	10
			Predator	288.9900	25.38374	10
			Sleep	283.1580	23.20535	10
			Combined	280.9140	26.09613	10
			Total	279.9180	25.76397	40

		Total	Control	258.3450	25.54495	20
			Predator	266.1800	30.88204	20
			Sleep	268.8755	28.60557	20
			Combined	260.5725	30.54428	20
			Total	263.4933	28.73196	80
	Total	SD	Control	338.0450	96.07687	20
			Predator	332.5200	95.40789	20
			Sleep	341.3835	97.71213	20
			Combined	330.1945	94.20118	20
			Total	335.5358	94.12532	80
		LE	Control	350.6800	91.08872	20
			Predator	373.4400	91.78489	20
			Sleep	351.5725	78.62706	20
			Combined	350.0090	79.56495	20
			Total	356.4254	84.43397	80
		Total	Control	344.3625	92.62925	40
			Predator	352.9800	94.70060	40
			Sleep	346.4780	87.69203	40
			Combined	340.1017	86.64851	40
			Total	345.9806	89.74317	160

Table 35. Multivariate ANOVA (Body Weight) - Tests of Between-Subjects Effects, Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
SEX	1st BW	99.194	1	99.194	1.507	.222	.010	.230
	2nd BW	5202.505	1	5202.505	45.708	.000	.241	1.000
	3rd BW	54563.337	1	54563.337	302.265	.000	.677	1.000
	4th BW	161448.789	1	161448.789	536.277	.000	.788	1.000
	5th BW	433849.324	1	433849.324	592.914	.000	.805	1.000
	6th BW	680038.614	1	680038.614	1042.472	.000	.879	1.000

	7th BW	929630.953	1	929630.953	1105.563	.000	.885	1.000
	8th BW	1088665.076	1	1088665.076	1014.798	.000	.876	1.000
STRAIN	1st BW	4484.865	1	4484.865	68.148	.000	.321	1.000
	2nd BW	3911.891	1	3911.891	34.369	.000	.193	1.000
	3rd BW	1206.263	1	1206.263	6.682	.011	.044	.728
	4th BW	161.765	1	161.765	.537	.465	.004	.113
	5th BW	2642.738	1	2642.738	3.612	.059	.024	.471
	6th BW	7333.535	1	7333.535	11.242	.001	.072	.915
	7th BW	14217.801	1	14217.801	16.909	.000	.105	.983
	8th BW	17455.057	1	17455.057	16.271	.000	.102	.980
CONDITION	1st BW	123.580	3	41.193	.626	.599	.013	.179
	2nd BW	331.835	3	110.612	.972	.408	.020	.261
	3rd BW	1184.222	3	394.741	2.187	.092	.044	.546
	4th BW	1446.248	3	482.083	1.601	.192	.032	.414
	5th BW	91.542	3	30.514	.042	.989	.001	.057
	6th BW	1410.671	3	470.224	.721	.541	.015	.201
	7th BW	809.004	3	269.668	.321	.810	.007	.111
	8th BW	3456.725	3	1152.242	1.074	.362	.022	.286
SEX * STRAIN	1st BW	910.307	1	910.307	13.832	.000	.088	.959
	2nd BW	224.439	1	224.439	1.972	.162	.014	.286
	3rd BW	229.824	1	229.824	1.273	.261	.009	.202
	4th BW	183.098	1	183.098	.608	.437	.004	.121
	5th BW	1827.228	1	1827.228	2.497	.116	.017	.348
	6th BW	3090.564	1	3090.564	4.738	.031	.032	.580
	7th BW	3352.561	1	3352.561	3.987	.048	.027	.509
	8th BW	5721.544	1	5721.544	5.333	.022	.036	.631
SEX * CONDITION	1st BW	122.090	3	40.697	.618	.604	.013	.177
	2nd BW	252.022	3	84.007	.738	.531	.015	.205
	3rd BW	935.095	3	311.698	1.727	.164	.035	.444
	4th BW	778.252	3	259.417	.862	.463	.018	.234
	5th BW	497.481	3	165.827	.227	.878	.005	.092
	6th BW	1360.253	3	453.418	.695	.556	.014	.195

	7th BW	3068.219	3	1022.740	1.216	.306	.025	.321
	8th BW	2546.921	3	848.974	.791	.501	.016	.217
STRAIN * CONDITION	1st BW	66.848	3	22.283	.339	.797	.007	.115
	2nd BW	403.623	3	134.541	1.182	.319	.024	.313
	3rd BW	775.492	3	258.497	1.432	.236	.029	.374
	4th BW	1651.979	3	550.660	1.829	.144	.037	.468
	5th BW	5192.142	3	1730.714	2.365	.073	.047	.583
	6th BW	4526.560	3	1508.853	2.313	.079	.046	.573
	7th BW	4943.806	3	1647.935	1.960	.123	.039	.497
	8th BW	5850.140	3	1950.047	1.818	.147	.036	.465
SEX * STRAIN * CONDITION	1st BW	324.162	3	108.054	1.642	.182	.033	.424
	2nd BW	577.712	3	192.571	1.692	.171	.034	.436
	3rd BW	684.612	3	228.204	1.264	.289	.026	.333
	4th BW	1355.774	3	451.925	1.501	.217	.030	.390
	5th BW	2377.737	3	792.579	1.083	.358	.022	.288
	6th BW	1487.290	3	495.763	.760	.518	.016	.210
	7th BW	1672.049	3	557.350	.663	.576	.014	.187
	8th BW	2382.783	3	794.261	.740	.530	.015	.205
Error	1st BW	9476.694	144	65.810				
	2nd BW	16390.296	144	113.822				
	3rd BW	25994.187	144	180.515				
	4th BW	43351.857	144	301.055				
	5th BW	105368.197	144	731.724				
	6th BW	93935.890	144	652.333				
	7th BW	121084.817	144	840.867				
	8th BW	154481.738	144	1072.790				

Table 36. Repeated-Measures ANOVA (Body Weight) - Tests of Within-Subjects Effects

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Week	1.057E7	7	1509923.771	8119.152	.000	.983	1.000
Week * SEX	1160613.373	7	165801.910	891.549	.000	.861	1.000
Week * STRAIN	44400.326	7	6342.904	34.107	.000	.191	1.000
Week * CONDITION	5435.183	21	258.818	1.392	.112	.028	.927
Week * SEX * STRAIN	9738.850	7	1391.264	7.481	.000	.049	1.000
Week * SEX * CONDITION	3706.973	21	176.523	.949	.526	.019	.754
Week * STRAIN * CONDITION	6074.481	21	289.261	1.555	.053	.031	.957
Week * SEX * STRAIN * CONDITION	2199.512	21	104.739	.563	.943	.012	.463
Error(Week)	187458.381	1008	185.971				

Table 37. Repeated-Measures ANOVA (Body Weight) –Tests of Between-Subjects Effects, Experiment 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Intercept	6.691E7	1	6.691E7	25181.075	.000	.994	1.000
SEX	2192884.418	1	2192884.418	825.286	.000	.851	1.000
STRAIN	7013.589	1	7013.589	2.640	.106	.018	.365
CONDITION	3418.643	3	1139.548	.429	.733	.009	.134
SEX * STRAIN	5800.716	1	5800.716	2.183	.142	.015	.312
SEX * CONDITION	5853.359	3	1951.120	.734	.533	.015	.204
STRAIN * CONDITION	17336.109	3	5778.703	2.175	.094	.043	.544

SEX * STRAIN * CONDITION	8662.606	3	2887.535	1.087	.357	.022	.289
Error	382625.295	144	2657.120				

Table 38. Post Hoc Analysis: Body Weight by Condition, Experiment 2

						95% Confidence Interval	
	(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	Lower Bound	Upper Bound
Tukey HSD	Control	Predator	-1.9569	4.07517	.963	-12.5494	8.6356
		Sleep	-2.4536	4.07517	.931	-13.0461	8.1389
		Combined	1.6535	4.07517	.977	-8.9391	12.2460
	Predator	Control	1.9569	4.07517	.963	-8.6356	12.5494
		Sleep	-.4967	4.07517	.999	-11.0892	10.0958
		Combined	3.6104	4.07517	.812	-6.9821	14.2029
	Sleep	Control	2.4536	4.07517	.931	-8.1389	13.0461
		Predator	.4967	4.07517	.999	-10.0958	11.0892
		Combined	4.1071	4.07517	.745	-6.4855	14.6996
	Combined	Control	-1.6535	4.07517	.977	-12.2460	8.9391
		Predator	-3.6104	4.07517	.812	-14.2029	6.9821
		Sleep	-4.1071	4.07517	.745	-14.6996	6.4855
Dunnett t (2-sided)	Control	Combined	1.6535	4.07517	.957	-8.0212	11.3281
	Predator	Combined	3.6104	4.07517	.705	-6.0643	13.2850
	Sleep	Combined	4.1071	4.07517	.620	-5.5676	13.7817

Table 39. Multivariate ANOVA (Male Body Weight), Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1st BW	1112.764[a]	7	158.966	2.377	.030	.188	.824
	2nd BW	2381.354[c]	7	340.193	2.541	.021	.198	.853
	3rd BW	4461.875[d]	7	637.411	2.706	.015	.208	.878

	4th BW	4547.635[e]	7	649.662	1.570	.158	.132	.614
	5th BW	7270.756[f]	7	1038.679	.895	.515	.080	.359
	6th BW	7112.823[g]	7	1016.118	1.110	.367	.097	.445
	7th BW	9534.917[h]	7	1362.131	1.117	.362	.098	.447
	8th BW	11861.266[i]	7	1694.467	1.063	.396	.094	.426
Intercept	1st BW	478717.606	1	478717.606	7157.586	.000	.990	1.000
	2nd BW	1367106.363	1	1367106.363	10211.693	.000	.993	1.000
	3rd BW	2973878.305	1	2973878.305	12624.042	.000	.994	1.000
	4th BW	4911336.584	1	4911336.584	11867.958	.000	.994	1.000
	5th BW	8034745.995	1	8034745.995	6922.582	.000	.990	1.000
	6th BW	1.050E7	1	1.050E7	11465.023	.000	.994	1.000
	7th BW	1.305E7	1	1.305E7	10696.848	.000	.993	1.000
	8th BW	1.469E7	1	1.469E7	9209.845	.000	.992	1.000
STRAIN	1st BW	677.041	1	677.041	10.123	.002	.123	.881
	2nd BW	1131.158	1	1131.158	8.449	.005	.105	.818
	3rd BW	1244.569	1	1244.569	5.283	.024	.068	.621
	4th BW	.330	1	.330	.001	.978	.000	.050
	5th BW	37.511	1	37.511	.032	.858	.000	.054
	6th BW	451.298	1	451.298	.493	.485	.007	.107
	7th BW	1881.121	1	1881.121	1.542	.218	.021	.232
	8th BW	1594.809	1	1594.809	1.000	.321	.014	.167
CONDITION	1st BW	100.343	3	33.448	.500	.683	.020	.147
	2nd BW	323.272	3	107.757	.805	.495	.032	.216
	3rd BW	1789.590	3	596.530	2.532	.064	.095	.603
	4th BW	1746.213	3	582.071	1.407	.248	.055	.358
	5th BW	346.195	3	115.398	.099	.960	.004	.067
	6th BW	1907.636	3	635.879	.694	.559	.028	.190
	7th BW	3062.398	3	1020.799	.837	.478	.034	.223
	8th BW	4579.196	3	1526.399	.957	.418	.038	.251
STRAIN * CONDITION	1st BW	335.381	3	111.794	1.671	.181	.065	.420
	2nd BW	926.923	3	308.974	2.308	.084	.088	.559
	3rd BW	1427.716	3	475.905	2.020	.119	.078	.499

	4th BW	2801.092	3	933.697	2.256	.089	.086	.548
	5th BW	6887.050	3	2295.683	1.978	.125	.076	.489
	6th BW	4753.890	3	1584.630	1.730	.168	.067	.434
	7th BW	4591.398	3	1530.466	1.255	.296	.050	.322
	8th BW	5687.261	3	1895.754	1.189	.320	.047	.306
Error	1st BW	4815.544	72	66.883				
	2nd BW	9639.112	72	133.877				
	3rd BW	16961.226	72	235.573				
	4th BW	29795.879	72	413.832				
	5th BW	83567.332	72	1160.657				
	6th BW	65940.030	72	915.834				
	7th BW	87826.893	72	1219.818				
	8th BW	114817.134	72	1594.682				

Table 40. Multivariate ANOVA (Female Body Weight), Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Corrected Model	1st BW	4919.087[a]	7	702.727	10.855	.000	.513	1.000
	2nd BW	3320.167[c]	7	474.310	5.058	.000	.330	.995
	3rd BW	553.632[d]	7	79.090	.630	.729	.058	.253
	4th BW	1029.482[e]	7	147.069	.781	.605	.071	.313
	5th BW	5358.112[f]	7	765.445	2.528	.022	.197	.851
	6th BW	12096.050[g]	7	1728.007	4.444	.000	.302	.987
	7th BW	18528.523[h]	7	2646.932	5.730	.000	.358	.998
	8th BW	25551.905[i]	7	3650.272	6.626	.000	.392	1.000
Intercept	1st BW	498406.642	1	498406.642	7698.803	.000	.991	1.000
	2nd BW	1138976.219	1	1138976.219	12146.949	.000	.994	1.000
	3rd BW	1943655.256	1	1943655.256	15492.505	.000	.995	1.000
	4th BW	2715616.535	1	2715616.535	14423.481	.000	.995	1.000
	5th BW	3621634.767	1	3621634.767	11960.888	.000	.994	1.000
	6th BW	4302120.678	1	4302120.678	11064.232	.000	.994	1.000
	7th BW	5056574.133	1	5056574.133	10946.965	.000	.993	1.000
	8th BW	5554295.424	1	5554295.424	10082.271	.000	.993	1.000

STRAIN	1st BW	4718.131	1	4718.131	72.880	.000	.503	1.000
	2nd BW	3005.171	1	3005.171	32.050	.000	.308	1.000
	3rd BW	191.519	1	191.519	1.527	.221	.021	.230
	4th BW	344.533	1	344.533	1.830	.180	.025	.266
	5th BW	4432.455	1	4432.455	14.639	.000	.169	.965
	6th BW	9972.801	1	9972.801	25.648	.000	.263	.999
	7th BW	15689.241	1	15689.241	33.966	.000	.321	1.000
	8th BW	21581.793	1	21581.793	39.176	.000	.352	1.000
CONDITION	1st BW	145.327	3	48.442	.748	.527	.030	.203
	2nd BW	260.584	3	86.861	.926	.433	.037	.244
	3rd BW	329.726	3	109.909	.876	.458	.035	.232
	4th BW	478.287	3	159.429	.847	.473	.034	.225
	5th BW	242.828	3	80.943	.267	.849	.011	.099
	6th BW	863.288	3	287.763	.740	.532	.030	.201
	7th BW	814.825	3	271.608	.588	.625	.024	.166
	8th BW	1424.450	3	474.817	.862	.465	.035	.229
STRAIN * CONDITION	1st BW	55.629	3	18.543	.286	.835	.012	.102
	2nd BW	54.412	3	18.137	.193	.901	.008	.084
	3rd BW	32.387	3	10.796	.086	.967	.004	.065
	4th BW	206.662	3	68.887	.366	.778	.015	.119
	5th BW	682.829	3	227.610	.752	.525	.030	.203
	6th BW	1259.961	3	419.987	1.080	.363	.043	.281
	7th BW	2024.457	3	674.819	1.461	.232	.057	.371
	8th BW	2545.662	3	848.554	1.540	.211	.060	.390
Error	1st BW	4661.151	72	64.738				
	2nd BW	6751.184	72	93.766				
	3rd BW	9032.960	72	125.458				
	4th BW	13555.978	72	188.277				
	5th BW	21800.865	72	302.790				
	6th BW	27995.860	72	388.831				
	7th BW	33257.923	72	461.916				
	8th BW	39664.604	72	550.897				

Table 41. Descriptives - Weekly Body Weight Gain, Experiment 2

	N	Mean	
	Statistic	Statistic	Std. Error
Wght Gain wk 1	160	46.8785	.69167
Wght Gain wk 2	160	49.3155	1.16526
Wght Gain wk 3	160	41.6704	1.22105
Wght Gain wk 4	160	47.5832	1.73611
Wght Gain wk 5	160	33.5004	1.33681
Wght Gain wk 6	160	30.5435	1.09829
Wght Gain wk 7	160	18.3456	.79902
Valid N (listwise)	160		

Table 42. Descriptives - Weekly Body Weight Gain by Sex, Experiment 2

Sex		N	Mean	
		Statistic	Statistic	Std. Error
Male	Wght Gain wk 1	80	53.3681	.67077
	Wght Gain wk 2	80	62.0800	.81542
	Wght Gain wk 3	80	54.9692	.90803
	Wght Gain wk 4	80	66.6402	1.31131
	Wght Gain wk 5	80	47.8716	1.08741
	Wght Gain wk 6	80	41.5742	.94778
	Wght Gain wk 7	80	24.6082	.81306
	Valid N (listwise)	80		
Female	Wght Gain wk 1	80	40.3889	.64001
	Wght Gain wk 2	80	36.5510	.82217
	Wght Gain wk 3	80	28.3715	.83642
	Wght Gain wk 4	80	28.5262	1.10406
	Wght Gain wk 5	80	19.1291	.88453
	Wght Gain wk 6	80	19.5128	.93638
	Wght Gain wk 7	80	12.0829	.95701
	Valid N (listwise)	80		

Table 43. Descriptives - Weekly Body Weight Gain by Strain, Experiment 2

		N	Mean	
		Statistic	Statistic	Std. Error
SD	Wght Gain wk 1	80	46.5288	1.07460
	Wght Gain wk 2	80	47.1166	1.83836
	Wght Gain wk 3	80	37.9191	1.63155
	Wght Gain wk 4	80	43.2746	2.48742
	Wght Gain wk 5	80	32.0444	2.05215
	Wght Gain wk 6	80	27.8870	1.50013
	Wght Gain wk 7	80	17.3274	1.15619
	Valid N (listwise)	80		
LE	Wght Gain wk 1	80	47.2282	.87629
	Wght Gain wk 2	80	51.5144	1.40135
	Wght Gain wk 3	80	45.4216	1.72724
	Wght Gain wk 4	80	51.8919	2.33996
	Wght Gain wk 5	80	34.9564	1.71129
	Wght Gain wk 6	80	33.2000	1.55771
	Wght Gain wk 7	80	19.3637	1.09856
	Valid N (listwise)	80		

Table 44. Descriptives - Weekly Body Weight Gain by Condition, Experiment 2

Condition		N	Mean	
		Statistic	Statistic	Std. Error
Control	Wght Gain wk 1	40	47.8100	1.26940
	Wght Gain wk 2	40	51.3050	2.40635
	Wght Gain wk 3	40	41.8400	2.42700
	Wght Gain wk 4	40	45.8275	3.26258
	Wght Gain wk 5	40	30.6350	2.81369
	Wght Gain wk 6	40	31.4725	2.34237
	Wght Gain wk 7	40	18.5850	1.76746
	Valid N (listwise)	40		

Predator	Wght Gain wk 1	40	45.3375	1.31799
	Wght Gain wk 2	40	48.2350	2.29961
	Wght Gain wk 3	40	40.9252	2.36529
	Wght Gain wk 4	40	52.4648	4.07666
	Wght Gain wk 5	40	34.6625	2.72920
	Wght Gain wk 6	40	30.8425	2.54584
	Wght Gain wk 7	40	21.6900	1.32539
	Valid N (listwise)	40		
Sleep	Wght Gain wk 1	40	48.3545	1.70904
	Wght Gain wk 2	40	50.4175	2.62209
	Wght Gain wk 3	40	42.1153	2.60441
	Wght Gain wk 4	40	44.9827	3.04408
	Wght Gain wk 5	40	34.6750	2.57721
	Wght Gain wk 6	40	28.0430	1.88510
	Wght Gain wk 7	40	18.7930	1.48623
	Valid N (listwise)	40		
Combined	Wght Gain wk 1	40	46.0120	1.17228
	Wght Gain wk 2	40	47.3045	1.98064
	Wght Gain wk 3	40	41.8010	2.45475
	Wght Gain wk 4	40	47.0580	3.42816
	Wght Gain wk 5	40	34.0290	2.61685
	Wght Gain wk 6	40	31.8160	1.98073
	Wght Gain wk 7	40	14.3143	1.61608
	Valid N (listwise)	40		

Table 45. Descriptives - Body Weight Gain (Sex by Strain by Condition), Experiment 2

Sex	Strain	Condition		N	Mean	
				Statistic	Statistic	Std. Error
Male	SD	Control	Wght Gain wk 1	10	55.6300	1.92735
			Wght Gain wk 2	10	63.0800	2.22994
			Wght Gain wk 3	10	53.6800	2.71239

			Wght Gain wk 4	10	59.8300	2.76446
			Wght Gain wk 5	10	44.6000	3.03465
			Wght Gain wk 6	10	41.2200	1.64329
			Wght Gain wk 7	10	27.4000	2.61653
			Valid N (listwise)	10		
		Predator	Wght Gain wk 1	10	50.7200	1.25112
			Wght Gain wk 2	10	60.2800	1.37944
			Wght Gain wk 3	10	46.4550	1.29546
			Wght Gain wk 4	10	69.2650	5.63141
			Wght Gain wk 5	10	49.8300	2.58492
			Wght Gain wk 6	10	41.7000	2.59863
			Wght Gain wk 7	10	24.7500	1.74631
			Valid N (listwise)	10		
		Sleep	Wght Gain wk 1	10	57.9190	2.74597
			Wght Gain wk 2	10	66.2230	3.45389
			Wght Gain wk 3	10	50.1920	1.92658
			Wght Gain wk 4	10	61.5580	3.69217
			Wght Gain wk 5	10	48.8340	3.61208
			Wght Gain wk 6	10	36.0820	2.56931
			Wght Gain wk 7	10	24.9690	2.06998
			Valid N (listwise)	10		
		Combined	Wght Gain wk 1	10	52.6080	1.20870
			Wght Gain wk 2	10	59.4730	1.19103
			Wght Gain wk 3	10	54.0300	1.86132
			Wght Gain wk 4	10	62.9120	1.68539
			Wght Gain wk 5	10	51.4610	2.43242
			Wght Gain wk 6	10	37.3990	1.52326
			Wght Gain wk 7	10	22.8510	2.13318
			Valid N (listwise)	10		
	LE	Control	Wght Gain wk 1	10	53.1200	1.32663
			Wght Gain wk 2	10	66.7000	1.69260
			Wght Gain wk 3	10	57.4300	1.89532

			Wght Gain wk 4	10	68.9700	2.64079
			Wght Gain wk 5	10	45.2800	4.62200
			Wght Gain wk 6	10	44.4600	3.84142
			Wght Gain wk 7	10	25.9500	2.34745
			Valid N (listwise)	10		
		Predator	Wght Gain wk 1	10	53.5100	1.73733
			Wght Gain wk 2	10	61.6200	2.11044
			Wght Gain wk 3	10	61.3140	1.69889
			Wght Gain wk 4	10	78.0560	2.53769
			Wght Gain wk 5	10	50.1900	2.40007
			Wght Gain wk 6	10	46.5900	3.11632
			Wght Gain wk 7	10	27.0000	2.72580
			Valid N (listwise)	10		
		Sleep	Wght Gain wk 1	10	53.3660	1.96935
			Wght Gain wk 2	10	63.0270	2.09135
			Wght Gain wk 3	10	59.6170	3.33951
			Wght Gain wk 4	10	61.6300	3.12993
			Wght Gain wk 5	10	47.5220	3.30252
			Wght Gain wk 6	10	39.5970	2.30252
			Wght Gain wk 7	10	22.8050	2.82536
			Valid N (listwise)	10		
		Combined	Wght Gain wk 1	10	50.0720	1.93076
			Wght Gain wk 2	10	56.2370	2.31388
			Wght Gain wk 3	10	57.0360	2.27234
			Wght Gain wk 4	10	70.9010	3.38963
			Wght Gain wk 5	10	45.2560	2.20981
			Wght Gain wk 6	10	45.5460	1.92943
			Wght Gain wk 7	10	21.1410	1.73467
			Valid N (listwise)	10		
Female	SD	Control	Wght Gain wk 1	10	40.3900	1.08530
			Wght Gain wk 2	10	33.3800	1.82092
			Wght Gain wk 3	10	26.0600	1.88345

			Wght Gain wk 4	10	22.1900	1.34656
			Wght Gain wk 5	10	14.5800	2.10949
			Wght Gain wk 6	10	18.5300	2.83659
			Wght Gain wk 7	10	10.1000	1.88526
			Valid N (listwise)	10		
		Predator	Wght Gain wk 1	10	35.9200	1.01037
			Wght Gain wk 2	10	30.0900	1.01592
			Wght Gain wk 3	10	23.9480	.87541
			Wght Gain wk 4	10	22.1620	1.28219
			Wght Gain wk 5	10	14.4300	1.91915
			Wght Gain wk 6	10	14.7500	2.51958
			Wght Gain wk 7	10	14.6300	2.18134
			Valid N (listwise)	10		
		Sleep	Wght Gain wk 1	10	39.1910	2.01560
			Wght Gain wk 2	10	32.7420	1.17296
			Wght Gain wk 3	10	26.0070	1.98857
			Wght Gain wk 4	10	24.5120	2.52841
			Wght Gain wk 5	10	17.6590	1.42710
			Wght Gain wk 6	10	17.3140	2.04301
			Wght Gain wk 7	10	9.7540	1.69267
			Valid N (listwise)	10		
		Combined	Wght Gain wk 1	10	39.8520	1.80066
			Wght Gain wk 2	10	31.6650	1.90131
			Wght Gain wk 3	10	22.9810	1.81716
			Wght Gain wk 4	10	23.7680	1.72891
			Wght Gain wk 5	10	14.9610	1.02299
			Wght Gain wk 6	10	16.1010	2.42050
			Wght Gain wk 7	10	4.1650	2.11076
			Valid N (listwise)	10		
	LE	Control	Wght Gain wk 1	10	42.1000	1.26903
			Wght Gain wk 2	10	42.0600	1.50954
			Wght Gain wk 3	10	30.1900	1.50750

			Wght Gain wk 4	10	32.3200	2.10390
			Wght Gain wk 5	10	18.0800	3.24797
			Wght Gain wk 6	10	21.6800	3.32174
			Wght Gain wk 7	10	10.8900	2.99954
			Valid N (listwise)	10		
		Predator	Wght Gain wk 1	10	41.2000	1.45205
			Wght Gain wk 2	10	40.9500	2.40159
			Wght Gain wk 3	10	31.9840	1.07111
			Wght Gain wk 4	10	40.3760	5.08660
			Wght Gain wk 5	10	24.2000	1.72794
			Wght Gain wk 6	10	20.3300	2.79726
			Wght Gain wk 7	10	20.3800	2.30409
			Valid N (listwise)	10		
		Sleep	Wght Gain wk 1	10	42.9420	3.09936
			Wght Gain wk 2	10	39.6780	2.92247
			Wght Gain wk 3	10	32.6450	4.32137
			Wght Gain wk 4	10	32.2310	2.20196
			Wght Gain wk 5	10	24.6850	2.29427
			Wght Gain wk 6	10	19.1790	1.40893
			Wght Gain wk 7	10	17.6440	2.81131
			Valid N (listwise)	10		
		Combined	Wght Gain wk 1	10	41.5160	1.50371
			Wght Gain wk 2	10	41.8430	1.34195
			Wght Gain wk 3	10	33.1570	1.56662
			Wght Gain wk 4	10	30.6510	2.26157
			Wght Gain wk 5	10	24.4380	2.90893
			Wght Gain wk 6	10	28.2180	1.67756
			Wght Gain wk 7	10	9.1000	2.34799
			Valid N (listwise)	10		

Table 46. Repeated-Measures ANOVA (Body Weight Gain) –

Tests of Within-Subjects Effects, Experiment 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Week	123814.312	6	20635.719	471.534	.000	.766	1.000
Week * SEX	19494.543	6	3249.090	74.243	.000	.340	1.000
Week * STRAIN	1986.924	6	331.154	7.567	.000	.050	1.000
Week * CONDITION	3558.530	18	197.696	4.517	.000	.086	1.000
Week * SEX * STRAIN	863.383	6	143.897	3.288	.003	.022	.935
Week * SEX * CONDITION	1508.091	18	83.783	1.914	.012	.038	.976
Week * STRAIN * CONDITION	911.365	18	50.631	1.157	.291	.024	.809
Week * SEX * STRAIN * CONDITION	696.286	18	38.683	.884	.599	.018	.660
Error(Week)	37811.197	864	43.763				

Table 47. Multivariate ANOVA (Body Weight Gain), Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
SEX	Wght Gain wk 1	6738.437	1	6738.437	208.316	.000	.591	1.000
	Wght Gain wk 2	26069.194	1	26069.194	641.736	.000	.817	1.000
	Wght Gain wk 3	28297.612	1	28297.612	602.683	.000	.807	1.000
	Wght Gain wk 4	58107.080	1	58107.080	647.299	.000	.818	1.000
	Wght Gain wk 5	33045.252	1	33045.252	454.838	.000	.760	1.000
	Wght Gain wk 6	19468.391	1	19468.391	311.643	.000	.684	1.000
	Wght Gain wk 7	6275.401	1	6275.401	116.726	.000	.448	1.000
STRAIN	Wght Gain wk 1	19.572	1	19.572	.605	.438	.004	.121
	Wght Gain wk 2	773.608	1	773.608	19.044	.000	.117	.991

	Wght Gain wk 3	2251.500	1	2251.500	47.952	.000	.250	1.000
	Wght Gain wk 4	2970.280	1	2970.280	33.088	.000	.187	1.000
	Wght Gain wk 5	339.190	1	339.190	4.669	.032	.031	.574
	Wght Gain wk 6	1129.119	1	1129.119	18.075	.000	.112	.988
	Wght Gain wk 7	165.873	1	165.873	3.085	.081	.021	.415
CONDITION	Wght Gain wk 1	246.871	3	82.290	2.544	.059	.050	.618
	Wght Gain wk 2	415.365	3	138.455	3.408	.019	.066	.759
	Wght Gain wk 3	31.958	3	10.653	.227	.878	.005	.092
	Wght Gain wk 4	1358.008	3	452.669	5.043	.002	.095	.912
	Wght Gain wk 5	448.804	3	149.601	2.059	.108	.041	.519
	Wght Gain wk 6	352.968	3	117.656	1.883	.135	.038	.480
	Wght Gain wk 7	1107.771	3	369.257	6.868	.000	.125	.975
SEX * STRAIN	Wght Gain wk 1	230.736	1	230.736	7.133	.008	.047	.756
	Wght Gain wk 2	908.495	1	908.495	22.364	.000	.134	.997
	Wght Gain wk 3	2.652	1	2.652	.056	.812	.000	.056
	Wght Gain wk 4	179.649	1	179.649	2.001	.159	.014	.290
	Wght Gain wk 5	821.289	1	821.289	11.304	.001	.073	.916
	Wght Gain wk 6	5.329	1	5.329	.085	.771	.001	.060
	Wght Gain wk 7	314.693	1	314.693	5.853	.017	.039	.671
SEX * CONDITION	Wght Gain wk 1	83.013	3	27.671	.855	.466	.018	.233
	Wght Gain wk 2	306.389	3	102.130	2.514	.061	.050	.612
	Wght Gain wk 3	29.433	3	9.811	.209	.890	.004	.088
	Wght Gain wk 4	456.686	3	152.229	1.696	.171	.034	.437
	Wght Gain wk 5	68.522	3	22.841	.314	.815	.007	.110
	Wght Gain wk 6	347.447	3	115.816	1.854	.140	.037	.473
	Wght Gain wk 7	441.417	3	147.139	2.737	.046	.054	.654
STRAIN * CONDITION	Wght Gain wk 1	148.349	3	49.450	1.529	.210	.031	.397
	Wght Gain wk 2	132.164	3	44.055	1.084	.358	.022	.289
	Wght Gain wk 3	293.651	3	97.884	2.085	.105	.042	.525
	Wght Gain wk 4	485.918	3	161.973	1.804	.149	.036	.462
	Wght Gain wk 5	69.423	3	23.141	.319	.812	.007	.111
	Wght Gain wk 6	345.949	3	115.316	1.846	.141	.037	.471

	Wght Gain wk 7	103.185	3	34.395	.640	.591	.013	.182
SEX * STRAIN * CONDITION	Wght Gain wk 1	45.776	3	15.259	.472	.702	.010	.144
	Wght Gain wk 2	88.572	3	29.524	.727	.538	.015	.202
	Wght Gain wk 3	262.033	3	87.344	1.860	.139	.037	.475
	Wght Gain wk 4	194.033	3	64.678	.720	.541	.015	.201
	Wght Gain wk 5	208.581	3	69.527	.957	.415	.020	.257
	Wght Gain wk 6	42.090	3	14.030	.225	.879	.005	.092
	Wght Gain wk 7	91.573	3	30.524	.568	.637	.012	.165
Error	Wght Gain wk 1	4657.992	144	32.347				
	Wght Gain wk 2	5849.702	144	40.623				
	Wght Gain wk 3	6761.195	144	46.953				
	Wght Gain wk 4	12926.674	144	89.769				
	Wght Gain wk 5	10462.013	144	72.653				
	Wght Gain wk 6	8995.709	144	62.470				
	Wght Gain wk 7	7741.693	144	53.762				

Table 48. Post Hoc Analysis (Body Weight Gain), Experiment 2

Dependent Variable		(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Wght Gain wk 2	Tukey HSD	Control	Predator	2.4725	1.27175	.214	-.8332	5.7782
			Sleep	-.5445	1.27175	.974	-3.8502	2.7612
			Combined	1.7980	1.27175	.493	-1.5077	5.1037
		Predator	Control	-2.4725	1.27175	.214	-5.7782	.8332
			Sleep	-3.0170	1.27175	.087	-6.3227	.2887
			Combined	-.6745	1.27175	.952	-3.9802	2.6312
		Sleep	Control	.5445	1.27175	.974	-2.7612	3.8502
			Predator	3.0170	1.27175	.087	-.2887	6.3227
			Combined	2.3425	1.27175	.258	-.9632	5.6482
		Combined	Control	-1.7980	1.27175	.493	-5.1037	1.5077

			Predator	.6745	1.27175	.952	-2.6312	3.9802
			Sleep	-2.3425	1.27175	.258	-5.6482	.9632
Wght Gain wk 3	Tukey HSD	Control	Predator	3.0700	1.42518	.141	-.6345	6.7745
			Sleep	.8875	1.42518	.925	-2.8170	4.5920
			Combined	4.0005	1.42518	.029	.2960	7.7050
		Predator	Control	-3.0700	1.42518	.141	-6.7745	.6345
			Sleep	-2.1825	1.42518	.422	-5.8870	1.5220
			Combined	.9305	1.42518	.914	-2.7740	4.6350
		Sleep	Control	-.8875	1.42518	.925	-4.5920	2.8170
			Predator	2.1825	1.42518	.422	-1.5220	5.8870
			Combined	3.1130	1.42518	.133	-.5915	6.8175
		Combined	Control	-4.0005	1.42518	.029	-7.7050	-.2960
			Predator	-.9305	1.42518	.914	-4.6350	2.7740
			Sleep	-3.1130	1.42518	.133	-6.8175	.5915
Wght Gain wk 4	Tukey HSD	Control	Predator	.9148	1.53220	.933	-3.0679	4.8974
			Sleep	-.2753	1.53220	.998	-4.2579	3.7074
			Combined	.0390	1.53220	1.000	-3.9436	4.0216
		Predator	Control	-.9148	1.53220	.933	-4.8974	3.0679
			Sleep	-1.1900	1.53220	.865	-5.1726	2.7926
			Combined	-.8758	1.53220	.940	-4.8584	3.1069
		Sleep	Control	.2753	1.53220	.998	-3.7074	4.2579
			Predator	1.1900	1.53220	.865	-2.7926	5.1726
			Combined	.3143	1.53220	.997	-3.6684	4.2969
		Combined	Control	-.0390	1.53220	1.000	-4.0216	3.9436
			Predator	.8758	1.53220	.940	-3.1069	4.8584
			Sleep	-.3143	1.53220	.997	-4.2969	3.6684
Wght Gain wk 5	Tukey HSD	Control	Predator	-6.6372	2.11859	.011	-12.1441	-1.1304
			Sleep	.8448	2.11859	.978	-4.6621	6.3516
			Combined	-1.2305	2.11859	.938	-6.7373	4.2763
		Predator	Control	6.6372	2.11859	.011	1.1304	12.1441
			Sleep	7.4820	2.11859	.003	1.9752	12.9888
			Combined	5.4067	2.11859	.056	-.1001	10.9136

		Sleep	Control	-.8448	2.11859	.978	-6.3516	4.6621
			Predator	-7.4820	2.11859	.003	-12.9888	-1.9752
			Combined	-2.0753	2.11859	.761	-7.5821	3.4316
		Combined	Control	1.2305	2.11859	.938	-4.2763	6.7373
			Predator	-5.4067	2.11859	.056	-10.9136	.1001
			Sleep	2.0753	2.11859	.761	-3.4316	7.5821
Wght Gain wk 6	Tukey HSD	Control	Predator	-4.0275	1.90595	.154	-8.9816	.9266
			Sleep	-4.0400	1.90595	.152	-8.9941	.9141
			Combined	-3.3940	1.90595	.287	-8.3481	1.5601
		Predator	Control	4.0275	1.90595	.154	-.9266	8.9816
			Sleep	-.0125	1.90595	1.000	-4.9666	4.9416
			Combined	.6335	1.90595	.987	-4.3206	5.5876
		Sleep	Control	4.0400	1.90595	.152	-.9141	8.9941
			Predator	.0125	1.90595	1.000	-4.9416	4.9666
			Combined	.6460	1.90595	.987	-4.3081	5.6001
		Combined	Control	3.3940	1.90595	.287	-1.5601	8.3481
			Predator	-.6335	1.90595	.987	-5.5876	4.3206
			Sleep	-.6460	1.90595	.987	-5.6001	4.3081
Wght Gain wk 7	Tukey HSD	Control	Predator	.6300	1.76735	.984	-3.9638	5.2238
			Sleep	3.4295	1.76735	.216	-1.1643	8.0233
			Combined	-.3435	1.76735	.997	-4.9373	4.2503
		Predator	Control	-.6300	1.76735	.984	-5.2238	3.9638
			Sleep	2.7995	1.76735	.391	-1.7943	7.3933
			Combined	-.9735	1.76735	.946	-5.5673	3.6203
		Sleep	Control	-3.4295	1.76735	.216	-8.0233	1.1643
			Predator	-2.7995	1.76735	.391	-7.3933	1.7943
			Combined	-3.7730	1.76735	.147	-8.3668	.8208
		Combined	Control	.3435	1.76735	.997	-4.2503	4.9373
			Predator	.9735	1.76735	.946	-3.6203	5.5673
			Sleep	3.7730	1.76735	.147	-.8208	8.3668
Wght Gain wk 8	Tukey HSD	Control	Predator	-3.1050	1.63954	.235	-7.3666	1.1566
			Sleep	-.2080	1.63954	.999	-4.4696	4.0536

			Combined	4.2708	1.63954	.049	.0091	8.5324
		Predator	Control	3.1050	1.63954	.235	-1.1566	7.3666
			Sleep	2.8970	1.63954	.294	-1.3646	7.1586
			Combined	7.3758	1.63954	.000	3.1141	11.6374
		Sleep	Control	.2080	1.63954	.999	-4.0536	4.4696
			Predator	-2.8970	1.63954	.294	-7.1586	1.3646
			Combined	4.4788	1.63954	.035	.2171	8.7404
		Combined	Control	-4.2708	1.63954	.049	-8.5324	-.0091
			Predator	-7.3758	1.63954	.000	-11.6374	-3.1141
			Sleep	-4.4788	1.63954	.035	-8.7404	-.2171

Table 49. Multivariate ANOVA (Food Consumption) – Tests of Between-Subjects Effects, Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
SEX	FC 2nd Week	15373.955	1	15373.955	21.711	.000	.266	.996
	FC 3rd Week	62381.711	1	62381.711	158.690	.000	.726	1.000
	FC 4th Week	125268.306	1	125268.306	86.644	.000	.591	1.000
	FC 5th Week	259995.568	1	259995.568	182.818	.000	.753	1.000
	FC 6th Week	243686.470	1	243686.470	114.162	.000	.655	1.000
	FC 7th Week	204868.842	1	204868.842	132.695	.000	.689	1.000
	FC 8th Week	198703.202	1	198703.202	209.938	.000	.778	1.000
STRAIN	FC 2nd Week	559.982	1	559.982	.791	.377	.013	.141
	FC 3rd Week	7963.176	1	7963.176	20.257	.000	.252	.993
	FC 4th Week	18028.797	1	18028.797	12.470	.001	.172	.935
	FC 5th Week	88695.475	1	88695.475	62.367	.000	.510	1.000
	FC 6th Week	7053.076	1	7053.076	3.304	.074	.052	.432
	FC 7th Week	11484.633	1	11484.633	7.439	.008	.110	.765
	FC 8th Week	20374.718	1	20374.718	21.527	.000	.264	.995
CONDITION	FC 2nd Week	3534.415	3	1178.138	1.664	.184	.077	.415
	FC 3rd Week	5537.581	3	1845.860	4.696	.005	.190	.876

	FC 4th Week	20175.535	3	6725.178	4.652	.005	.189	.873
	FC 5th Week	13328.987	3	4442.996	3.124	.032	.135	.699
	FC 6th Week	3750.477	3	1250.159	.586	.627	.028	.164
	FC 7th Week	8362.607	3	2787.536	1.806	.156	.083	.447
	FC 8th Week	1203.780	3	401.260	.424	.737	.021	.130
SEX * STRAIN	FC 2nd Week	37.557	1	37.557	.053	.819	.001	.056
	FC 3rd Week	1047.082	1	1047.082	2.664	.108	.043	.362
	FC 4th Week	639.685	1	639.685	.442	.508	.007	.100
	FC 5th Week	6125.110	1	6125.110	4.307	.042	.067	.533
	FC 6th Week	7.568	1	7.568	.004	.953	.000	.050
	FC 7th Week	7.467	1	7.467	.005	.945	.000	.051
	FC 8th Week	4827.175	1	4827.175	5.100	.028	.078	.603
SEX * CONDITION	FC 2nd Week	4048.550	3	1349.517	1.906	.138	.087	.469
	FC 3rd Week	1150.972	3	383.657	.976	.410	.047	.253
	FC 4th Week	806.627	3	268.876	.186	.906	.009	.083
	FC 5th Week	4559.317	3	1519.772	1.069	.369	.051	.275
	FC 6th Week	17534.268	3	5844.756	2.738	.051	.120	.635
	FC 7th Week	5983.728	3	1994.576	1.292	.285	.061	.328
	FC 8th Week	297.649	3	99.216	.105	.957	.005	.068
STRAIN * CONDITION	FC 2nd Week	1493.386	3	497.795	.703	.554	.034	.190
	FC 3rd Week	2856.653	3	952.218	2.422	.075	.108	.577
	FC 4th Week	809.247	3	269.749	.187	.905	.009	.083
	FC 5th Week	11108.979	3	3702.993	2.604	.060	.115	.611
	FC 6th Week	17006.551	3	5668.850	2.656	.056	.117	.621
	FC 7th Week	11684.739	3	3894.913	2.523	.066	.112	.596
	FC 8th Week	4319.844	3	1439.948	1.521	.218	.071	.382
SEX * STRAIN * CONDITION	FC 2nd Week	4409.528	3	1469.843	2.076	.113	.094	.506
	FC 3rd Week	1959.852	3	653.284	1.662	.185	.077	.414
	FC 4th Week	17188.085	3	5729.362	3.963	.012	.165	.809
	FC 5th Week	1327.246	3	442.415	.311	.817	.015	.107
	FC 6th Week	21861.222	3	7287.074	3.414	.023	.146	.742
	FC 7th Week	16398.590	3	5466.197	3.540	.020	.150	.759

	FC 8th Week	2431.649	3	810.550	.856	.469	.041	.225
Error	FC 2nd Week	42486.250	60	708.104				
	FC 3rd Week	23586.295	60	393.105				
	FC 4th Week	86747.029	60	1445.784				
	FC 5th Week	85329.510	60	1422.158				
	FC 6th Week	128073.663	60	2134.561				
	FC 7th Week	92634.362	60	1543.906				
	FC 8th Week	56789.161	60	946.486				

Table 50. Multivariate ANOVA - Center Time/Horizontal Activity Ratio, Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
SEX	CT_HRZ_Ratio_BL	.001	1	.001	4.215	.042	.028	.532
	CT_HRZ_Ratio_PS	.000	1	.000	1.241	.267	.009	.198
	CT_HRZ_Ratio_Adult	.015	1	.015	25.456	.000	.150	.999
	CT_HRZ_Ratio_Novel	.026	1	.026	33.523	.000	.189	1.000
STRAIN	CT_HRZ_Ratio_BL	.000	1	.000	1.084	.299	.007	.179
	CT_HRZ_Ratio_PS	.000	1	.000	.697	.405	.005	.132
	CT_HRZ_Ratio_Adult	2.391E-5	1	2.391E-5	.040	.842	.000	.055
	CT_HRZ_Ratio_Novel	.005	1	.005	6.527	.012	.043	.718
CONDITION	CT_HRZ_Ratio_BL	.001	3	.000	1.315	.272	.027	.345
	CT_HRZ_Ratio_PS	.002	3	.001	1.656	.179	.033	.427
	CT_HRZ_Ratio_Adult	.002	3	.001	1.219	.305	.025	.322
	CT_HRZ_Ratio_Novel	.006	3	.002	2.649	.051	.052	.638
SEX * STRAIN	CT_HRZ_Ratio_BL	.000	1	.000	1.351	.247	.009	.211
	CT_HRZ_Ratio_PS	.002	1	.002	4.293	.040	.029	.539
	CT_HRZ_Ratio_Adult	.001	1	.001	1.386	.241	.010	.215
	CT_HRZ_Ratio_Novel	.002	1	.002	2.519	.115	.017	.351
SEX * CONDITION	CT_HRZ_Ratio_BL	.001	3	.000	1.428	.237	.029	.373
	CT_HRZ_Ratio_PS	.001	3	.000	1.157	.328	.024	.307
	CT_HRZ_Ratio_Adult	.002	3	.001	.928	.429	.019	.251
	CT_HRZ_Ratio_Novel	.001	3	.000	.551	.648	.011	.161
STRAIN * CONDITION	CT_HRZ_Ratio_BL	.000	3	8.362E-5	.557	.644	.011	.163
	CT_HRZ_Ratio_PS	.001	3	.000	.626	.599	.013	.179
	CT_HRZ_Ratio_Adult	.001	3	.000	.339	.797	.007	.115
	CT_HRZ_Ratio_Novel	.000	3	.000	.165	.920	.003	.080
SEX * STRAIN * CONDITION	CT_HRZ_Ratio_BL	.000	3	5.062E-5	.337	.798	.007	.115
	CT_HRZ_Ratio_PS	.000	3	5.872E-5	.158	.924	.003	.079
	CT_HRZ_Ratio_Adult	.000	3	.000	.184	.907	.004	.084
	CT_HRZ_Ratio_Novel	.003	3	.001	1.265	.289	.026	.333

Error	CT_HRZ_Ratio_BL	.022	144	.000				
	CT_HRZ_Ratio_PS	.053	144	.000				
	CT_HRZ_Ratio_Adult	.086	144	.001				
	CT_HRZ_Ratio_Novel	.112	144	.001				

Table 51. Post Hoc Analysis, Center Time/Horizontal Activity Ratio, Experiment 2

Dependent Variable	(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CTR_HRZ_ Ratio_BL	Control	Predator	.0039	.00274	.495	-.0033	.0110
		Sleep	.0044	.00274	.386	-.0028	.0115
		Combined	.0049	.00274	.288	-.0022	.0120
	Predator	Control	-.0039	.00274	.495	-.0110	.0033
		Sleep	.0005	.00274	.998	-.0066	.0076
		Combined	.0010	.00274	.983	-.0061	.0081
	Sleep	Control	-.0044	.00274	.386	-.0115	.0028
		Predator	-.0005	.00274	.998	-.0076	.0066
		Combined	.0005	.00274	.998	-.0066	.0076
	Combined	Control	-.0049	.00274	.288	-.0120	.0022
		Predator	-.0010	.00274	.983	-.0081	.0061
		Sleep	-.0005	.00274	.998	-.0076	.0066
CTR_HRZ_ Ratio_PS	Control	Predator	.0004	.00430	1.000	-.0108	.0116
		Sleep	.0048	.00430	.674	-.0063	.0160
		Combined	.0082	.00430	.227	-.0030	.0194
	Predator	Control	-.0004	.00430	1.000	-.0116	.0108
		Sleep	.0044	.00430	.731	-.0067	.0156
		Combined	.0078	.00430	.268	-.0034	.0190
	Sleep	Control	-.0048	.00430	.674	-.0160	.0063
		Predator	-.0044	.00430	.731	-.0156	.0067
		Combined	.0034	.00430	.860	-.0078	.0146
	Combined	Control	-.0082	.00430	.227	-.0194	.0030
		Predator	-.0078	.00430	.268	-.0190	.0034
		Sleep	-.0034	.00430	.860	-.0146	.0078

CTR_HRZ_ Ratio_Adult	Control	Predator	.0040	.00546	.881	-.0102	.0182
		Sleep	.0082	.00546	.443	-.0060	.0224
		Combined	.0094	.00546	.318	-.0048	.0236
	Predator	Control	-.0040	.00546	.881	-.0182	.0102
		Sleep	.0041	.00546	.874	-.0101	.0183
		Combined	.0053	.00546	.762	-.0089	.0195
	Sleep	Control	-.0082	.00546	.443	-.0224	.0060
		Predator	-.0041	.00546	.874	-.0183	.0101
		Combined	.0012	.00546	.996	-.0130	.0154
	Combined	Control	-.0094	.00546	.318	-.0236	.0048
		Predator	-.0053	.00546	.762	-.0195	.0089
		Sleep	-.0012	.00546	.996	-.0154	.0130
CTR_HRZ_ Ratio_Novel	Control	Predator	.0075	.00623	.625	-.0087	.0237
		Sleep	.0107	.00623	.316	-.0055	.0269
		Combined	.0172*	.00623	.032	.0011	.0334
	Predator	Control	-.0075	.00623	.625	-.0237	.0087
		Sleep	.0032	.00623	.955	-.0130	.0194
		Combined	.0097	.00623	.402	-.0064	.0259
	Sleep	Control	-.0107	.00623	.316	-.0269	.0055
		Predator	-.0032	.00623	.955	-.0194	.0130
		Combined	.0065	.00623	.722	-.0097	.0227
	Combined	Control	-.0172*	.00623	.032	-.0334	-.0011
		Predator	-.0097	.00623	.402	-.0259	.0064
		Sleep	-.0065	.00623	.722	-.0227	.0097

Table 52. Descriptives - Forced Swim Immobility (in seconds), Experiment 2

	N	Mean	
	Statistic	Statistic	Std. Error
Baseline Immobile	160	77.2025	7.02406
Post Stress Immobile	160	16.8387	2.02302
Adult Immobile	160	22.5644	2.44640
Novel Stress Immobile	160	19.5994	2.55448
Valid N (listwise)	160		

Table 53. Descriptives - Forced Swim Immobility by Sex, Experiment 2

Sex		N	Mean	
		Statistic	Statistic	Std. Error
Male	Baseline Immobile	80	96.4325	11.75130
	Post Stress Immobile	80	24.8350	3.55939
	Adult Immobile	80	35.0625	4.22919
	Novel Stress Immobile	80	31.7612	4.44639
	Valid N (listwise)	80		
Female	Baseline Immobile	80	57.9725	7.15149
	Post Stress Immobile	80	8.8425	1.47853
	Adult Immobile	80	10.0662	1.50012
	Novel Stress Immobile	80	7.4375	1.65883
	Valid N (listwise)	80		

Table 54. Descriptives - Forced Swim Immobility by Strain, Experiment 2

Strain		N	Mean	
		Statistic	Statistic	Std. Error
SD	Baseline Immobile	80	128.5313	9.13430
	Post Stress Immobile	80	31.6587	3.28102
	Adult Immobile	80	41.2362	3.83589

	Novel Stress Immobile	80	36.6713	4.33163
	Valid N (listwise)	80		
LE	Baseline Immobile	80	25.8737	6.96157
	Post Stress Immobile	80	2.0188	.38548
	Adult Immobile	80	3.8925	.74205
	Novel Stress Immobile	80	2.5275	.35387
	Valid N (listwise)	80		

Table 55. Descriptives - Forced Swim Immobility by Sex and Strain, Experiment 2

Strain	Sex		N	Mean	
			Statistic	Statistic	Std. Error
SD	Male	Baseline Immobile	40	162.7300	13.41887
		Post Stress Immobile	40	46.8425	5.10296
		Adult Immobile	40	63.8900	5.29287
		Novel Stress Immobile	40	59.7950	6.27941
		Valid N (listwise)	40		
	Female	Baseline Immobile	40	94.3325	9.89835
		Post Stress Immobile	40	16.4750	2.39778
		Adult Immobile	40	18.5825	2.29752
		Novel Stress Immobile	40	13.5475	3.02692
		Valid N (listwise)	40		
LE	Male	Baseline Immobile	40	30.1350	12.40906
		Post Stress Immobile	40	2.8275	.67006
		Adult Immobile	40	6.2350	1.35292
		Novel Stress Immobile	40	3.7275	.60179
		Valid N (listwise)	40		
	Female	Baseline Immobile	40	21.6125	6.43608
		Post Stress Immobile	40	1.2100	.34567
		Adult Immobile	40	1.5500	.34495
		Novel Stress Immobile	40	1.3275	.26703
		Valid N (listwise)	40		

Table 56. Descriptives - Forced Swim Immobility by Condition, Experiment 2

Condition		N	Mean	
		Statistic	Statistic	Std. Error
Control	Baseline Immobile	40	71.8600	11.97820
	Post Stress Immobile	40	16.6650	4.01443
	Adult Immobile	40	18.3450	3.64397
	Novel Stress Immobile	40	12.2625	2.77759
	Valid N (listwise)	40		
Predator	Baseline Immobile	40	89.6800	16.65467
	Post Stress Immobile	40	15.1125	2.93512
	Adult Immobile	40	20.9575	3.86888
	Novel Stress Immobile	40	19.9625	4.97888
	Valid N (listwise)	40		
Sleep	Baseline Immobile	40	82.2400	14.82277
	Post Stress Immobile	40	21.3150	5.55758
	Adult Immobile	40	29.0625	6.78912
	Novel Stress Immobile	40	28.7825	7.29695
	Valid N (listwise)	40		
Combined	Baseline Immobile	40	65.0300	12.44330
	Post Stress Immobile	40	14.2625	3.21908
	Adult Immobile	40	21.8925	4.65225
	Novel Stress Immobile	40	17.3900	4.12225
	Valid N (listwise)	40		

Table 57. Descriptives, Forced Swim Immobility (Sex, Strain and Condition) Experiment 2

Sex	Strain	Condition		N	Mean	
				Statistic	Statistic	Std. Error
Male	SD	Control	Baseline Immobile	10	156.8500	19.10952
			Post Stress Immobile	10	47.8700	9.41159
			Adult Immobile	10	48.4700	8.30942
			Novel Stress Immobile	10	33.1600	7.15845
			Valid N (listwise)	10		
		Predator	Baseline Immobile	10	170.1500	23.23168
			Post Stress Immobile	10	34.9900	5.88706
			Adult Immobile	10	57.8900	4.56663
			Novel Stress Immobile	10	65.7900	10.48310
			Valid N (listwise)	10		
		Sleep	Baseline Immobile	10	188.8500	32.19273
			Post Stress Immobile	10	65.8500	14.72099
			Adult Immobile	10	86.3200	14.83546
			Novel Stress Immobile	10	89.2100	15.15006
			Valid N (listwise)	10		
		Combined	Baseline Immobile	10	135.0700	31.76656
			Post Stress Immobile	10	38.6600	6.93633
			Adult Immobile	10	62.8800	9.44245
			Novel Stress Immobile	10	51.0200	10.26141
			Valid N (listwise)	10		
	LE	Control	Baseline Immobile	10	23.8000	10.40649
			Post Stress Immobile	10	4.3000	1.92913
			Adult Immobile	10	5.3000	1.18030
			Novel Stress Immobile	10	4.4100	1.33045
			Valid N (listwise)	10		
		Predator	Baseline Immobile	10	59.7700	48.41465
			Post Stress Immobile	10	2.5800	1.17225
			Adult Immobile	10	8.1400	3.93317

			Novel Stress Immobile	10	4.0600	1.29110
			Valid N (listwise)	10		
		Sleep	Baseline Immobile	10	19.9000	7.15652
			Post Stress Immobile	10	2.9400	1.33610
			Adult Immobile	10	6.3200	2.84167
			Novel Stress Immobile	10	2.3200	1.25094
			Valid N (listwise)	10		
		Combined	Baseline Immobile	10	17.0700	5.73887
			Post Stress Immobile	10	1.4900	.67354
			Adult Immobile	10	5.1800	2.48278
			Novel Stress Immobile	10	4.1200	.98700
			Valid N (listwise)	10		
Female	SD	Control	Baseline Immobile	10	82.1700	19.54101
			Post Stress Immobile	10	13.4900	5.76704
			Adult Immobile	10	17.3800	3.17829
			Novel Stress Immobile	10	11.0000	3.11048
			Valid N (listwise)	10		
		Predator	Baseline Immobile	10	97.8900	21.69784
			Post Stress Immobile	10	21.8900	4.94219
			Adult Immobile	10	15.9100	3.10073
			Novel Stress Immobile	10	8.2700	2.07632
			Valid N (listwise)	10		
		Sleep	Baseline Immobile	10	103.8800	20.75179
			Post Stress Immobile	10	14.3600	3.36020
			Adult Immobile	10	22.4900	7.55122
			Novel Stress Immobile	10	22.0900	10.96461
			Valid N (listwise)	10		
		Combined	Baseline Immobile	10	93.3900	19.67026
			Post Stress Immobile	10	16.1600	5.09163
			Adult Immobile	10	18.5500	3.47957
			Novel Stress Immobile	10	12.8300	3.55272
			Valid N (listwise)	10		

	LE	Control	Baseline Immobile	10	24.6200	17.68112
			Post Stress Immobile	10	1.0000	.45923
			Adult Immobile	10	2.2300	.95836
			Novel Stress Immobile	10	.4800	.24757
			Valid N (listwise)	10		
		Predator	Baseline Immobile	10	30.9100	15.59651
			Post Stress Immobile	10	.9900	.50143
			Adult Immobile	10	1.8900	.68758
			Novel Stress Immobile	10	1.7300	.47959
			Valid N (listwise)	10		
		Sleep	Baseline Immobile	10	16.3300	7.79630
			Post Stress Immobile	10	2.1100	1.15782
			Adult Immobile	10	1.1200	.67211
			Novel Stress Immobile	10	1.5100	.67929
			Valid N (listwise)	10		
		Combined	Baseline Immobile	10	14.5900	9.06394
			Post Stress Immobile	10	.7400	.37865
			Adult Immobile	10	.9600	.30991
			Novel Stress Immobile	10	1.5900	.61164
			Valid N (listwise)	10		

Table 58. Multivariate ANOVA (Forced Swim Immobility), Experiment 2

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
SEX	BL Immobile	59166.864	1	59166.864	12.051	.001	.077	.932
	Post Stress Immobile	10230.402	1	10230.402	33.254	.000	.188	1.000
	Adult Immobile	24992.501	1	24992.501	76.747	.000	.348	1.000
	Novel Stress Immobile	23665.793	1	23665.793	58.264	.000	.288	1.000
STRAIN	BL Immobile	421542.492	1	421542.492	85.856	.000	.374	1.000
	Post Stress Immobile	35141.184	1	35141.184	114.225	.000	.442	1.000
	Adult Immobile	55782.227	1	55782.227	171.296	.000	.543	1.000

	Novel Stress Immobile	46631.827	1	46631.827	114.805	.000	.444	1.000
CONDITION	BL Immobile	14311.059	3	4770.353	.972	.408	.020	.261
	Post Stress Immobile	1187.360	3	395.787	1.286	.281	.026	.338
	Adult Immobile	2522.489	3	840.830	2.582	.056	.051	.625
	Novel Stress Immobile	5726.909	3	1908.970	4.700	.004	.089	.890
SEX * STRAIN	BL Immobile	35850.156	1	35850.156	7.302	.008	.048	.766
	Post Stress Immobile	8265.625	1	8265.625	26.867	.000	.157	.999
	Adult Immobile	16501.875	1	16501.875	50.674	.000	.260	1.000
	Novel Stress Immobile	19226.033	1	19226.033	47.333	.000	.247	1.000
SEX * CONDITION	BL Immobile	4508.114	3	1502.705	.306	.821	.006	.108
	Post Stress Immobile	2053.406	3	684.469	2.225	.088	.044	.554
	Adult Immobile	1545.704	3	515.235	1.582	.196	.032	.410
	Novel Stress Immobile	2672.492	3	890.831	2.193	.091	.044	.548
STRAIN * CONDITION	BL Immobile	9226.057	3	3075.352	.626	.599	.013	.179
	Post Stress Immobile	857.349	3	285.783	.929	.429	.019	.251
	Adult Immobile	2748.514	3	916.171	2.813	.041	.055	.667
	Novel Stress Immobile	6200.639	3	2066.880	5.089	.002	.096	.914
SEX * STRAIN * CONDITION	BL Immobile	3515.869	3	1171.956	.239	.869	.005	.094
	Post Stress Immobile	2079.237	3	693.079	2.253	.085	.045	.560
	Adult Immobile	1268.231	3	422.744	1.298	.277	.026	.341
	Novel Stress Immobile	3391.269	3	1130.423	2.783	.043	.055	.662
Error	BL Immobile	707022.688	144	4909.880				
	Post Stress Immobile	44301.256	144	307.648				
	Adult Immobile	46893.327	144	325.648				
	Novel Stress Immobile	58490.409	144	406.183				

Table 59. Post Hoc Analysis (Forced Swim Immobility), Experiment 2

Dependent Variable	(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Baseline Immobile	Control	Predator	-17.8200	15.66825	.667	-58.5462	22.9062
		Sleep	-10.3800	15.66825	.911	-51.1062	30.3462
		Combined	6.8300	15.66825	.972	-33.8962	47.5562
	Predator	Control	17.8200	15.66825	.667	-22.9062	58.5462
		Sleep	7.4400	15.66825	.965	-33.2862	48.1662
		Combined	24.6500	15.66825	.397	-16.0762	65.3762
	Sleep	Control	10.3800	15.66825	.911	-30.3462	51.1062
		Predator	-7.4400	15.66825	.965	-48.1662	33.2862
		Combined	17.2100	15.66825	.691	-23.5162	57.9362
	Combined	Control	-6.8300	15.66825	.972	-47.5562	33.8962
		Predator	-24.6500	15.66825	.397	-65.3762	16.0762
		Sleep	-17.2100	15.66825	.691	-57.9362	23.5162
Post Stress Immobile	Control	Predator	1.5525	3.92204	.979	-8.6420	11.7470
		Sleep	-4.6500	3.92204	.637	-14.8445	5.5445
		Combined	2.4025	3.92204	.928	-7.7920	12.5970
	Predator	Control	-1.5525	3.92204	.979	-11.7470	8.6420
		Sleep	-6.2025	3.92204	.392	-16.3970	3.9920
		Combined	.8500	3.92204	.996	-9.3445	11.0445
	Sleep	Control	4.6500	3.92204	.637	-5.5445	14.8445
		Predator	6.2025	3.92204	.392	-3.9920	16.3970
		Combined	7.0525	3.92204	.278	-3.1420	17.2470
	Combined	Control	-2.4025	3.92204	.928	-12.5970	7.7920
		Predator	-.8500	3.92204	.996	-11.0445	9.3445
		Sleep	-7.0525	3.92204	.278	-17.2470	3.1420
Adult Immobile	Control	Predator	-2.6125	4.03515	.916	-13.1010	7.8760
		Sleep	-10.7175	4.03515	.043	-21.2060	-.2290

		Combined	-3.5475	4.03515	.816	-14.0360	6.9410
	Predator	Control	2.6125	4.03515	.916	-7.8760	13.1010
		Sleep	-8.1050	4.03515	.190	-18.5935	2.3835
		Combined	-.9350	4.03515	.996	-11.4235	9.5535
	Sleep	Control	10.7175	4.03515	.043	.2290	21.2060
		Predator	8.1050	4.03515	.190	-2.3835	18.5935
		Combined	7.1700	4.03515	.289	-3.3185	17.6585
	Combined	Control	3.5475	4.03515	.816	-6.9410	14.0360
		Predator	.9350	4.03515	.996	-9.5535	11.4235
		Sleep	-7.1700	4.03515	.289	-17.6585	3.3185
Novel Stress Immobile	Control	Predator	-7.7000	4.50657	.323	-19.4139	4.0139
		Sleep	-16.5200	4.50657	.002	-28.2339	-4.8061
		Combined	-5.1275	4.50657	.667	-16.8414	6.5864
	Predator	Control	7.7000	4.50657	.323	-4.0139	19.4139
		Sleep	-8.8200	4.50657	.209	-20.5339	2.8939
		Combined	2.5725	4.50657	.941	-9.1414	14.2864
	Sleep	Control	16.5200	4.50657	.002	4.8061	28.2339
		Predator	8.8200	4.50657	.209	-2.8939	20.5339
		Combined	11.3925	4.50657	.060	-.3214	23.1064
	Combined	Control	5.1275	4.50657	.667	-6.5864	16.8414
		Predator	-2.5725	4.50657	.941	-14.2864	9.1414
		Sleep	-11.3925	4.50657	.060	-23.1064	.3214

Table 60. Multivariate ANOVA Split by Strain (Forced Swim Immobility), Experiment 2

Strain	Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
SD	SEX	BL Immobile	93564.360	1	93564.360	16.200	.000	.184	.978
		Post Stress Immobile	18443.701	1	18443.701	30.551	.000	.298	1.000
		Adult Immobile	41055.391	1	41055.391	67.305	.000	.483	1.000
		Novel Stress Immobile	42776.625	1	42776.625	53.239	.000	.425	1.000
	CONDITION	BL Immobile	12681.554	3	4227.185	.732	.536	.030	.199

		Post Stress Immobile	2014.186	3	671.395	1.112	.350	.044	.288
		Adult Immobile	5231.352	3	1743.784	2.859	.043	.106	.662
		Novel Stress Immobile	11915.062	3	3971.687	4.943	.004	.171	.897
	SEX * CONDITION	BL Immobile	5214.306	3	1738.102	.301	.825	.012	.105
		Post Stress Immobile	4111.621	3	1370.540	2.270	.088	.086	.551
		Adult Immobile	2786.240	3	928.747	1.523	.216	.060	.386
		Novel Stress Immobile	6039.307	3	2013.102	2.505	.066	.095	.598
	Error	BL Immobile	415851.631	72	5775.717				
		Post Stress Immobile	43465.985	72	603.694				
		Adult Immobile	43919.621	72	609.995				
		Novel Stress Immobile	57851.149	72	803.488				
LE	SEX	BL Immobile	1452.660	1	1452.660	.359	.551	.005	.091
		Post Stress Immobile	52.326	1	52.326	4.510	.037	.059	.554
		Adult Immobile	438.984	1	438.984	10.629	.002	.129	.896
		Novel Stress Immobile	115.200	1	115.200	12.975	.001	.153	.944
	CONDITION	BL Immobile	10855.561	3	3618.520	.895	.448	.036	.237
		Post Stress Immobile	30.523	3	10.174	.877	.457	.035	.232
		Adult Immobile	39.650	3	13.217	.320	.811	.013	.109
		Novel Stress Immobile	12.486	3	4.162	.469	.705	.019	.140
	SEX * CONDITION	BL Immobile	2809.676	3	936.559	.232	.874	.010	.092
		Post Stress Immobile	21.021	3	7.007	.604	.615	.025	.170
		Adult Immobile	27.695	3	9.232	.224	.880	.009	.090

		Novel Stress Immobile	24.454	3	8.151	.918	.437	.037	.242
	Error	BL Immobile	291171.057	72	4044.042				
		Post Stress Immobile	835.271	72	11.601				
		Adult Immobile	2973.706	72	41.301				
		Novel Stress Immobile	639.260	72	8.879				

Table 61. ANOVA – CONDITION (Forced Swim Immobility by Strain and Sex), Experiment 2

Strain	Sex	Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
SD	Male	Corrected Model	Post Stress Immobility	5697.759	3	1899.253	1.958	.138
			Adult Immobility	7779.014	3	2593.005	2.599	.067
			Novel Stress Immobility	16876.061	3	5625.354	4.537	.008
		CONDITION	Post Stress Immobility	5697.759	3	1899.253	1.958	.138
			Adult Immobility	7779.014	3	2593.005	2.599	.067
			Novel Stress Immobility	16876.061	3	5625.354	4.537	.008
		Error	Post Stress Immobility	34925.019	36	970.139		
			Adult Immobility	35923.622	36	997.878		
			Novel Stress Immobility	44636.378	36	1239.899		
	Female	Corrected Model	Post Stress Immobility	428.049	3	142.683	.601	.618
			Adult Immobility	238.579	3	79.526	.358	.784
			Novel Stress Immobility	1078.309	3	359.436	.979	.413
		CONDITION	Post Stress Immobility	428.049	3	142.683	.601	.618

			Adult Immobility	238.579	3	79.526	.358	.784
			Novel Stress Immobility	1078.309	3	359.436	.979	.413
		Error	Post Stress Immobility	8540.966	36	237.249		
			Adult Immobility	7995.999	36	222.111		
			Novel Stress Immobility	13214.771	36	367.077		
LE	Male	Corrected Model	Post Stress Immobility	40.311	3	13.437	.733	.539
			Adult Immobility	56.235	3	18.745	.241	.867
			Novel Stress Immobility	27.115	3	9.038	.605	.616
		CONDITION	Post Stress Immobility	40.311	3	13.437	.733	.539
			Adult Immobility	56.235	3	18.745	.241	.867
			Novel Stress Immobility	27.115	3	9.038	.605	.616
		Error	Post Stress Immobility	660.109	36	18.336		
			Adult Immobility	2799.196	36	77.755		
			Novel Stress Immobility	537.845	36	14.940		
	Female	Corrected Model	Post Stress Immobility	11.234	3	3.745	.770	.519
			Adult Immobility	11.110	3	3.703	.764	.522
			Novel Stress Immobility	9.825	3	3.275	1.163	.337
		CONDITION	Post Stress Immobility	11.234	3	3.745	.770	.519
			Adult Immobility	11.110	3	3.703	.764	.522
			Novel Stress Immobility	9.825	3	3.275	1.163	.337
		Error	Post Stress Immobility	175.162	36	4.866		

			Adult Immobility	174.510	36	4.848		
			Novel Stress Immobility	101.415	36	2.817		

Table 62. Post Hoc Analysis (Forced Swim Immobility by Strain and Sex), Experiment 2

Strain	Sex	Dependent Variable		(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
									Lower Bound	Upper Bound
SD	Male	Post Stress Immobility	Tukey HSD	Control	Predator	12.8800	13.92939	.792	-24.6350	50.3950
					Sleep	-17.9800	13.92939	.575	-55.4950	19.5350
					Combined	9.2100	13.92939	.911	-28.3050	46.7250
				Predator	Control	-12.8800	13.92939	.792	-50.3950	24.6350
					Sleep	-30.8600	13.92939	.138	-68.3750	6.6550
					Combined	-3.6700	13.92939	.993	-41.1850	33.8450
				Sleep	Control	17.9800	13.92939	.575	-19.5350	55.4950
					Predator	30.8600	13.92939	.138	-6.6550	68.3750
					Combined	27.1900	13.92939	.225	-10.3250	64.7050
				Combined	Control	-9.2100	13.92939	.911	-46.7250	28.3050
					Predator	3.6700	13.92939	.993	-33.8450	41.1850
					Sleep	-27.1900	13.92939	.225	-64.7050	10.3250
		Adult Immobility	Tukey HSD	Control	Predator	-9.4200	14.12713	.909	-47.4676	28.6276
					Sleep	-37.8500	14.12713	.052	-75.8976	.1976
					Combined	-14.4100	14.12713	.739	-52.4576	23.6376
				Predator	Control	9.4200	14.12713	.909	-28.6276	47.4676
					Sleep	-28.4300	14.12713	.202	-66.4776	9.6176
					Combined	-4.9900	14.12713	.985	-43.0376	33.0576
				Sleep	Control	37.8500	14.12713	.052	-.1976	75.8976
					Predator	28.4300	14.12713	.202	-9.6176	66.4776
					Combined	23.4400	14.12713	.360	-14.6076	61.4876
				Combined	Control	14.4100	14.12713	.739	-23.6376	52.4576
					Predator	4.9900	14.12713	.985	-33.0576	43.0376

					Sleep	-23.4400	14.12713	.360	-61.4876	14.6076
		Novel Stress Immobility	Tukey HSD	Control	Predator	-32.6300	15.74738	.182	-75.0413	9.7813
					Sleep	-56.0500	15.74738	.006	-98.4613	-13.6387
					Combined	-17.8600	15.74738	.671	-60.2713	24.5513
				Predator	Control	32.6300	15.74738	.182	-9.7813	75.0413
					Sleep	-23.4200	15.74738	.455	-65.8313	18.9913
					Combined	14.7700	15.74738	.785	-27.6413	57.1813
				Sleep	Control	56.0500	15.74738	.006	13.6387	98.4613
					Predator	23.4200	15.74738	.455	-18.9913	65.8313
					Combined	38.1900	15.74738	.090	-4.2213	80.6013
				Combined	Control	17.8600	15.74738	.671	-24.5513	60.2713
					Predator	-14.7700	15.74738	.785	-57.1813	27.6413
					Sleep	-38.1900	15.74738	.090	-80.6013	4.2213
	Female	Post Stress Immobility	Tukey HSD	Control	Predator	-8.4000	6.88838	.619	-26.9520	10.1520
					Sleep	-.8700	6.88838	.999	-19.4220	17.6820
					Combined	-2.6700	6.88838	.980	-21.2220	15.8820
				Predator	Control	8.4000	6.88838	.619	-10.1520	26.9520
					Sleep	7.5300	6.88838	.696	-11.0220	26.0820
					Combined	5.7300	6.88838	.839	-12.8220	24.2820
				Sleep	Control	.8700	6.88838	.999	-17.6820	19.4220
					Predator	-7.5300	6.88838	.696	-26.0820	11.0220
					Combined	-1.8000	6.88838	.994	-20.3520	16.7520
				Combined	Control	2.6700	6.88838	.980	-15.8820	21.2220
					Predator	-5.7300	6.88838	.839	-24.2820	12.8220
					Sleep	1.8000	6.88838	.994	-16.7520	20.3520
		Adult Immobility	Tukey HSD	Control	Predator	1.4700	6.66500	.996	-16.4804	19.4204
					Sleep	-5.1100	6.66500	.869	-23.0604	12.8404
					Combined	-1.1700	6.66500	.998	-19.1204	16.7804
				Predator	Control	-1.4700	6.66500	.996	-19.4204	16.4804
					Sleep	-6.5800	6.66500	.758	-24.5304	11.3704
					Combined	-2.6400	6.66500	.979	-20.5904	15.3104

				Sleep	Control	5.1100	6.66500	.869	-12.8404	23.0604
					Predator	6.5800	6.66500	.758	-11.3704	24.5304
					Combined	3.9400	6.66500	.934	-14.0104	21.8904
				Combined	Control	1.1700	6.66500	.998	-16.7804	19.1204
					Predator	2.6400	6.66500	.979	-15.3104	20.5904
					Sleep	-3.9400	6.66500	.934	-21.8904	14.0104
		Novel Stress Immobility	Tukey HSD	Control	Predator	2.7300	8.56828	.989	-20.3463	25.8063
					Sleep	-11.0900	8.56828	.572	-34.1663	11.9863
					Combined	-1.8300	8.56828	.996	-24.9063	21.2463
				Predator	Control	-2.7300	8.56828	.989	-25.8063	20.3463
					Sleep	-13.8200	8.56828	.384	-36.8963	9.2563
					Combined	-4.5600	8.56828	.951	-27.6363	18.5163
				Sleep	Control	11.0900	8.56828	.572	-11.9863	34.1663
					Predator	13.8200	8.56828	.384	-9.2563	36.8963
					Combined	9.2600	8.56828	.703	-13.8163	32.3363
				Combined	Control	1.8300	8.56828	.996	-21.2463	24.9063
					Predator	4.5600	8.56828	.951	-18.5163	27.6363
					Sleep	-9.2600	8.56828	.703	-32.3363	13.8163
LE	Male	Post Stress Immobility	Tukey HSD	Control	Predator	1.7200	1.91501	.806	-3.4376	6.8776
					Sleep	1.3600	1.91501	.892	-3.7976	6.5176
					Combined	2.8100	1.91501	.467	-2.3476	7.9676
				Predator	Control	-1.7200	1.91501	.806	-6.8776	3.4376
					Sleep	-.3600	1.91501	.998	-5.5176	4.7976
					Combined	1.0900	1.91501	.941	-4.0676	6.2476
				Sleep	Control	-1.3600	1.91501	.892	-6.5176	3.7976
					Predator	.3600	1.91501	.998	-4.7976	5.5176
					Combined	1.4500	1.91501	.873	-3.7076	6.6076
				Combined	Control	-2.8100	1.91501	.467	-7.9676	2.3476
					Predator	-1.0900	1.91501	.941	-6.2476	4.0676
					Sleep	-1.4500	1.91501	.873	-6.6076	3.7076
		Adult Immobility	Tukey HSD	Control	Predator	-2.8400	3.94349	.888	-13.4607	7.7807
					Sleep	-1.0200	3.94349	.994	-11.6407	9.6007

					Combined	.1200	3.94349	1.000	-10.5007	10.7407
				Predator	Control	2.8400	3.94349	.888	-7.7807	13.4607
					Sleep	1.8200	3.94349	.967	-8.8007	12.4407
					Combined	2.9600	3.94349	.876	-7.6607	13.5807
				Sleep	Control	1.0200	3.94349	.994	-9.6007	11.6407
					Predator	-1.8200	3.94349	.967	-12.4407	8.8007
					Combined	1.1400	3.94349	.991	-9.4807	11.7607
				Combined	Control	-.1200	3.94349	1.000	-10.7407	10.5007
					Predator	-2.9600	3.94349	.876	-13.5807	7.6607
					Sleep	-1.1400	3.94349	.991	-11.7607	9.4807
		Novel Stress Immobility	Tukey HSD	Control	Predator	.3500	1.72859	.997	-4.3055	5.0055
					Sleep	2.0900	1.72859	.625	-2.5655	6.7455
					Combined	.2900	1.72859	.998	-4.3655	4.9455
				Predator	Control	-.3500	1.72859	.997	-5.0055	4.3055
					Sleep	1.7400	1.72859	.747	-2.9155	6.3955
					Combined	-.0600	1.72859	1.000	-4.7155	4.5955
				Sleep	Control	-2.0900	1.72859	.625	-6.7455	2.5655
					Predator	-1.7400	1.72859	.747	-6.3955	2.9155
					Combined	-1.8000	1.72859	.727	-6.4555	2.8555
				Combined	Control	-.2900	1.72859	.998	-4.9455	4.3655
					Predator	.0600	1.72859	1.000	-4.5955	4.7155
					Sleep	1.8000	1.72859	.727	-2.8555	6.4555
	Female	Post Stress Immobility	Tukey HSD	Control	Predator	.0100	.98647	1.000	-2.6468	2.6668
					Sleep	-1.1100	.98647	.677	-3.7668	1.5468
					Combined	.2600	.98647	.993	-2.3968	2.9168
				Predator	Control	-.0100	.98647	1.000	-2.6668	2.6468
					Sleep	-1.1200	.98647	.670	-3.7768	1.5368
					Combined	.2500	.98647	.994	-2.4068	2.9068
				Sleep	Control	1.1100	.98647	.677	-1.5468	3.7668
					Predator	1.1200	.98647	.670	-1.5368	3.7768
					Combined	1.3700	.98647	.514	-1.2868	4.0268
				Combined	Control	-.2600	.98647	.993	-2.9168	2.3968

					Predator	-.2500	.98647	.994	-2.9068	2.4068
					Sleep	-1.3700	.98647	.514	-4.0268	1.2868
		Adult Immobility	Tukey HSD	Control	Predator	.3400	.98463	.986	-2.3118	2.9918
					Sleep	1.1100	.98463	.675	-1.5418	3.7618
					Combined	1.2700	.98463	.575	-1.3818	3.9218
				Predator	Control	-.3400	.98463	.986	-2.9918	2.3118
					Sleep	.7700	.98463	.862	-1.8818	3.4218
					Combined	.9300	.98463	.781	-1.7218	3.5818
				Sleep	Control	-1.1100	.98463	.675	-3.7618	1.5418
					Predator	-.7700	.98463	.862	-3.4218	1.8818
					Combined	.1600	.98463	.998	-2.4918	2.8118
				Combined	Control	-1.2700	.98463	.575	-3.9218	1.3818
					Predator	-.9300	.98463	.781	-3.5818	1.7218
					Sleep	-.1600	.98463	.998	-2.8118	2.4918
		Novel Stress Immobility	Tukey HSD	Control	Predator	-1.2500	.75061	.356	-3.2716	.7716
					Sleep	-1.0300	.75061	.524	-3.0516	.9916
					Combined	-1.1100	.75061	.460	-3.1316	.9116
				Predator	Control	1.2500	.75061	.356	-.7716	3.2716
					Sleep	.2200	.75061	.991	-1.8016	2.2416
					Combined	.1400	.75061	.998	-1.8816	2.1616
				Sleep	Control	1.0300	.75061	.524	-.9916	3.0516
					Predator	-.2200	.75061	.991	-2.2416	1.8016
					Combined	-.0800	.75061	1.000	-2.1016	1.9416
				Combined	Control	1.1100	.75061	.460	-.9116	3.1316
					Predator	-.1400	.75061	.998	-2.1616	1.8816
					Sleep	.0800	.75061	1.000	-1.9416	2.1016

Table 63. Repeated-Measures ANOVA - Test of Between-Subjects Effects (EtOH Consumption), Experiment 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power
Intercept	52.559	1	52.559	73.075	.000	.558	73.075	1.000
SEX	2.538	1	2.538	3.529	.065	.057	3.529	.455
STRAIN	16.587	1	16.587	23.062	.000	.284	23.062	.997
CONDITION	8.571	3	2.857	3.972	.012	.170	11.917	.809
SEX * STRAIN	1.865	1	1.865	2.592	.113	.043	2.592	.353
SEX * CONDITION	6.940	3	2.313	3.217	.029	.143	9.650	.712
STRAIN * CONDITION	7.887	3	2.629	3.655	.018	.159	10.965	.772
SEX * STRAIN * CONDITION	10.679	3	3.560	4.949	.004	.204	14.848	.893
Error	41.716	58	.719					

Table 64. Repeated-Measures ANOVA (EtOH Consumption) – Tests of Within-Subjects Effects, Experiment 2

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Percent	17.141	2	8.570	13.995	.000	.194	.998
Percent * SEX	1.909	2	.955	1.559	.215	.026	.325
Percent * STRAIN	5.122	2	2.561	4.182	.018	.067	.726
Percent * CONDITION	7.031	6	1.172	1.914	.084	.090	.688
Percent * SEX * STRAIN	.599	2	.299	.489	.615	.008	.129
Percent * SEX * CONDITION	4.763	6	.794	1.296	.264	.063	.492
Percent * STRAIN * CONDITION	8.484	6	1.414	2.309	.038	.107	.782

Percent * SEX * STRAIN * CONDITION	6.609	6	1.101	1.799	.105	.085	.656
Error(Percent)	71.036	116	.612				

Table 65. Repeated-Measures ANOVA (EtOH Consumption)

Post Hoc Analysis, Experiment 2

(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Predator	.0085	.15686	.957	-.3055	.3225
	Sleep	-.3422	.16347	.041	-.6694	-.0149
	Combined	-.0914	.16105	.572	-.4138	.2309
Predator	Control	-.0085	.15686	.957	-.3225	.3055
	Sleep	-.3507	.16152	.034	-.6740	-.0274
	Combined	-.1000	.15908	.532	-.4184	.2185
Sleep	Control	.3422	.16347	.041	.0149	.6694
	Predator	.3507	.16152	.034	.0274	.6740
	Combined	.2507	.16560	.135	-.0808	.5822
Combined	Control	.0914	.16105	.572	-.2309	.4138
	Predator	.1000	.15908	.532	-.2185	.4184
	Sleep	-.2507	.16560	.135	-.5822	.0808

Figure 66. Repeated-Measures ANOVA (EtOH by Sex) –

Tests of Between-Subjects Effects, Experiment 2

Sex	Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power
Male	Intercept	38.733	1	38.733	46.421	.000	.615	1.000
	STRAIN	14.649	1	14.649	17.557	.000	.377	.982
	CONDITION	14.160	3	4.720	5.657	.004	.369	.913
	STRAIN * CONDITION	17.374	3	5.791	6.941	.001	.418	.961

	Error	24.197	29	.834				
Female	Intercept	16.151	1	16.151	26.735	.000	.480	.999
	STRAIN	3.699	1	3.699	6.124	.019	.174	.667
	CONDITION	.446	3	.149	.246	.864	.025	.091
	STRAIN * CONDITION	.294	3	.098	.162	.921	.017	.076
	Error	17.519	29	.604				

Figure 67. Repeated-Measures ANOVA (EtOH by Sex) Post Hoc Analysis

Sex	(I) Condition	(J) Condition	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Male	Control	Predator	.0949	.24231	.698	-.4007	.5905
		Sleep	-.5730	.25626	.033	-1.0972	-.0489
		Combined	-.0079	.24231	.974	-.5035	.4877
	Predator	Control	-.0949	.24231	.698	-.5905	.4007
		Sleep	-.6679	.25016	.012	-1.1796	-.1563
		Combined	-.1028	.23585	.666	-.5851	.3796
	Sleep	Control	.5730	.25626	.033	.0489	1.0972
		Predator	.6679	.25016	.012	.1563	1.1796
		Combined	.5652	.25016	.032	.0535	1.0768
	Combined	Control	.0079	.24231	.974	-.4877	.5035
		Predator	.1028	.23585	.666	-.3796	.5851
		Sleep	-.5652	.25016	.032	-1.0768	-.0535
Female	Control	Predator	-.0713	.20068	.725	-.4818	.3391
		Sleep	-.1376	.20618	.510	-.5593	.2841
		Combined	-.1731	.21286	.423	-.6084	.2622
	Predator	Control	.0713	.20068	.725	-.3391	.4818
		Sleep	-.0663	.20618	.750	-.4880	.3554
		Combined	-.1018	.21286	.636	-.5371	.3336
	Sleep	Control	.1376	.20618	.510	-.2841	.5593
		Predator	.0663	.20618	.750	-.3554	.4880

		Combined	-.0355	.21805	.872	-.4814	.4105
	Combined	Control	.1731	.21286	.423	-.2622	.6084
		Predator	.1018	.21286	.636	-.3336	.5371
		Sleep	.0355	.21805	.872	-.4105	.4814

APPENDIX B

A Brief History of Stress

A Brief History

Claude Bernard. Stress work began from a primarily biological basis. Although L. J. Henderson and Walter Cannon, both faculty at Harvard Medical School, are credited with the United States' inaugural work leading to stress research, their ideas were extensions of the work developed by a famous French physician and researcher considered to be the father of modern physiology—Claude Bernard. Bernard is credited with discovering the glycogenic function of the liver, the pancreatic involvement in digestion, the vasomotor regulation of body temperature, the physiologic effects of curare and carbon monoxide, and the vagal regulation of cardiac function (Gross, 1998). Bernard's ideas regarding the constancy of the internal environment, or "milieu interne" (Cannon, 1929, p. 399), developed from his study of the vasomotor regulation of bodily functions. He posited that external variations in the environment were compensated for by the organism in order to preserve internal stability in the internal environment, thereby preserving life (Gross, 1998), the seminal idea behind what would be later known as "homeostasis" (Cannon, 1929).

Walter B. Cannon. Among Walter Cannon's most meaningful contributions to physiology were the concepts of "fight or flight" (W. B. Cannon, 1915) and homeostasis (W. B. Cannon, 1929). Cannon's early work investigated the influence of emotional stimuli on bodily organs and systems (B. Cannon, 1994) and the function of the sympathetic nervous system and adrenal medulla in responses to distress. His initial studies identified many effects of distress, such as: altered facial expressions, increased heart rate, higher blood pressure,

mobilization of glucose from the liver, increased respiration rate, and redistribution of blood flow to the brain, lungs, and muscles. Cannon recognized that these responses, which occurred in response to environmental challenges, served a purpose for the organism—fight or flight. The fight or flight response was thought to prepare the organism for defense or escape as a means of survival.

Cannon defined homeostasis as “the coordinated physiological reactions which maintain most of the steady states in the body” (1929, p. 400). Homeostasis extended the work of Claude Bernard as an explanation of an organism, particularly higher organisms, to maintain a constant internal state despite environmental fluctuation (Gross, 1998). Cannon identified the role of the sympathetic nervous system to maintain homeostasis within certain parameters, and he also discovered that extreme environmental challenges disrupted this balance and placed the organism at risk. Cannon identified serious health consequences as a result of internal extremes, resulting in loss of homeostatic balance in body temperature, blood glucose levels, sodium chloride levels, and water (W. B. Cannon, 1929). High or low extremes of any of these physiological measures might be detrimental to the organism. Neither Bernard nor Cannon considered individual differences in their research.

Hans Selye. Further extending the biological perspective of stress, Hans Selye led the next generation of research by focusing on chronic response to stress, rather than the acute response pioneered by Cannon (B. Cannon, 1994). Selye was a young physician and medical laboratory scientist searching for new

female hormones when he first studied the stress response in 1935 (Viner, 1999). He began with simple injections of noxious agents to elicit stressful responses and then expanded his manipulations to cold exposure, excessive exercise, and administration of various drugs (e.g., atropine, morphine, formaldehyde) in non-lethal doses. He found that a predictable syndrome appeared which was independent of the pharmacological effects of the specific drugs administered, but which was more related to the response of damage on the organism inflicted by the substance administration. Selye named this response the "General Adaptation Syndrome," a nonspecific syndrome in response to a specific stressor (Selye, 1936, 1946).

The General Adaptation Syndrome (GAS) proposed by Selye was characterized by activation of the Hypothalmic-Pituitary-Adrenal (HPA) axis in response to stressors and occurred in three stages: Alarm, Resistance, and Exhaustion. Alarm and Resistance described an organism's initial, adaptive response to an environmental stressor, with the HPA axis responding to the period of distress and promoting adaptation (i.e., alarm to resistance) and restoring homeostasis. Prolonged exposure to stressful circumstances, however, overwhelmed the organism's capacity to adapt and resulted in "diseases of adaptation" including peptic ulcer, hypertension, and arthritis (Selye, 1956).

Following World War II, Selye was an expert consultant on stress to the Surgeon General of the Army from 1947 to 1957, lecturing at military academies including the Naval Medical School located in Bethesda, Maryland (Viner, 1999). Stress research interested the military establishment because of its potential use

as a weapon against the enemy as well as something against which to inoculate American troops, maximizing operational efficiency (Viner, 1999).

Selye speculated that individual characteristics including genetics, gender, age, drug treatments, past experiences, diet, climate, etc., might influence individual response to stressors or stress vulnerability (Selye, 1975). However, he did not conduct research to support his assertions.

Richard Lazarus. Whereas much of the stress research on stress had been focused on physiological stress responses (i.e., Bernard, Cannon, and Selye), Lazarus and his colleagues took psychological variables into account, focusing on individual differences (Lazarus et al., 1952). This line of research was inspired by post World War II observations that conditions of regular life, such as marriage, school exams, illness, etc., could produce responses to stress comparable to those of combat (Lazarus, 1993). The military wanted to be able to predict who might be stress resistant so that they could be trained to manage stress, but researchers found that stressors did not produce dependable effects. Under identically stressful conditions, the performance of some individuals improved, whereas others were impaired, and still others exhibited no change (Lazarus & Eriksen, 1952).

To explain differential individual responses to stress, Lazarus advocated for the consideration of four concepts: (1) a causal external or internal agent; (2) evaluation or appraisal of the threatening and benign; (3) coping processes; and (4) the stress reaction—a complex array of psychological and physiological effects (Lazarus, 1993).

John W. Mason. John Mason's perspective on stress can be considered a psychobiological approach, integrating several of the previously discussed concepts. Mason was concerned with the influence of psychological stress on the organism, and he believed that there were individual differences in stress response based on individual history, personality factors, coping style, and perception, which result in various behavioral and physiological responses (Mason, 1975). Working at the Walter Reed Institute of Research (WRAIR) in the 1950s - 1970s, Mason used psychologically-mediated HPA axis activation, to observe that there were marked individual differences in response to psychological factors such as predictability or control of the environment, coping mechanisms, personal history, or individual role (Bourne et al., 1967; Mason, 1968a-e; Poe et al., 1970; Hofer et al., 1972a, 1972b). Behavioral scientists such as Holmes and Rahe (1967) were able to quantify life stressors and demonstrate that stressful changes might be used to predict the development of later illness. Thomas (1977) supported the use of psychological testing and other criteria to predict the likelihood of suffering mental illness, hypertension, heart disease, or cancer in studies of medical students at Johns Hopkins.

David Glass and Jerome E. Singer. Predictability and controllability of the stressors in the environment are psychologically-mediated concepts that affect stress responses. Glass and Singer (1972) reported that individuals with perceived control (but not actual control) over stressors, such as electric shock and loud noise, rapidly adapted and exhibited near normal responses on measures of stress, such as galvanic skin response (GSR) and vasoconstriction.

Complex task performance also was affected by predictability and controllability. Generally, individuals who perceived that they had no control over noise and electric shock performed more poorly than those who perceived that they could control the noise or terminate the shock.

Bruce McEwen. The most modern and integrative conceptualization of stress is presented by Bruce McEwen. McEwen states that it is virtually impossible to separate behavior from biology because of the integral role that behavior plays as the environment alters biology, or as biological mediators, such as hormones underlie behaviors (McEwen, 2001a). McEwen's concept of stress as adaptive in the short term and maladaptive in the long term is similar to Selye's concepts of resistance and exhaustion (Selye, 1936, 1946). However, McEwen presents a far more detailed and complex account of major life stressors and his approach is reminiscent of Mason's psychobiological integration. According to McEwen, a process termed "allostasis" occurs in order to maintain internal stability (homeostasis) in response to immediate, short-term stressors (McEwen, 1998; Sterling & Eyer, 1988). He holds that two common mediators of allostasis, cortisol and adrenaline, are generally adaptive and promote adaptation when released in response to stressors such as restricted diet, sleep deprivation, and exercise. When these mediators do not shut down, stressors cease, do not respond appropriately to stress, or are overly taxed in response to multiple stressors, the cumulative effects produce wear and tear on the body and brain, an effect McEwen termed allostatic load (1998). If additional factors such as unpredictable events, disease, disturbance, social interactions,

and other stressors are added once there is allostatic load, then allostatic overload can occur, serving no functional purpose and predisposing the individual to disease (McEwen, 2004). Allostatic load and allostatic overload are affected by genetic factors, developmental factors, and behaviors of the individual (McEwen, 1998).

McEwen's conception of stress is germane to the current research because of its consideration of the short term, adaptive function of stress and the long-term, maladaptive effects of stress—directly addressing the types of stress faced by deployed military personnel who are often exposed to repeated acute stressors as well as to chronic stressors. McEwen's perspective also considers individual differences in biology and experience, an issue identified after World War II as relevant to understanding how to best treat and prepare military personnel for the challenges they might face. This modern perspective of stress provides rationale for the key stressors manipulated in this study, predator stress and sleep disruption, both of which have implications for allostatic load.

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